FIGURE 1A

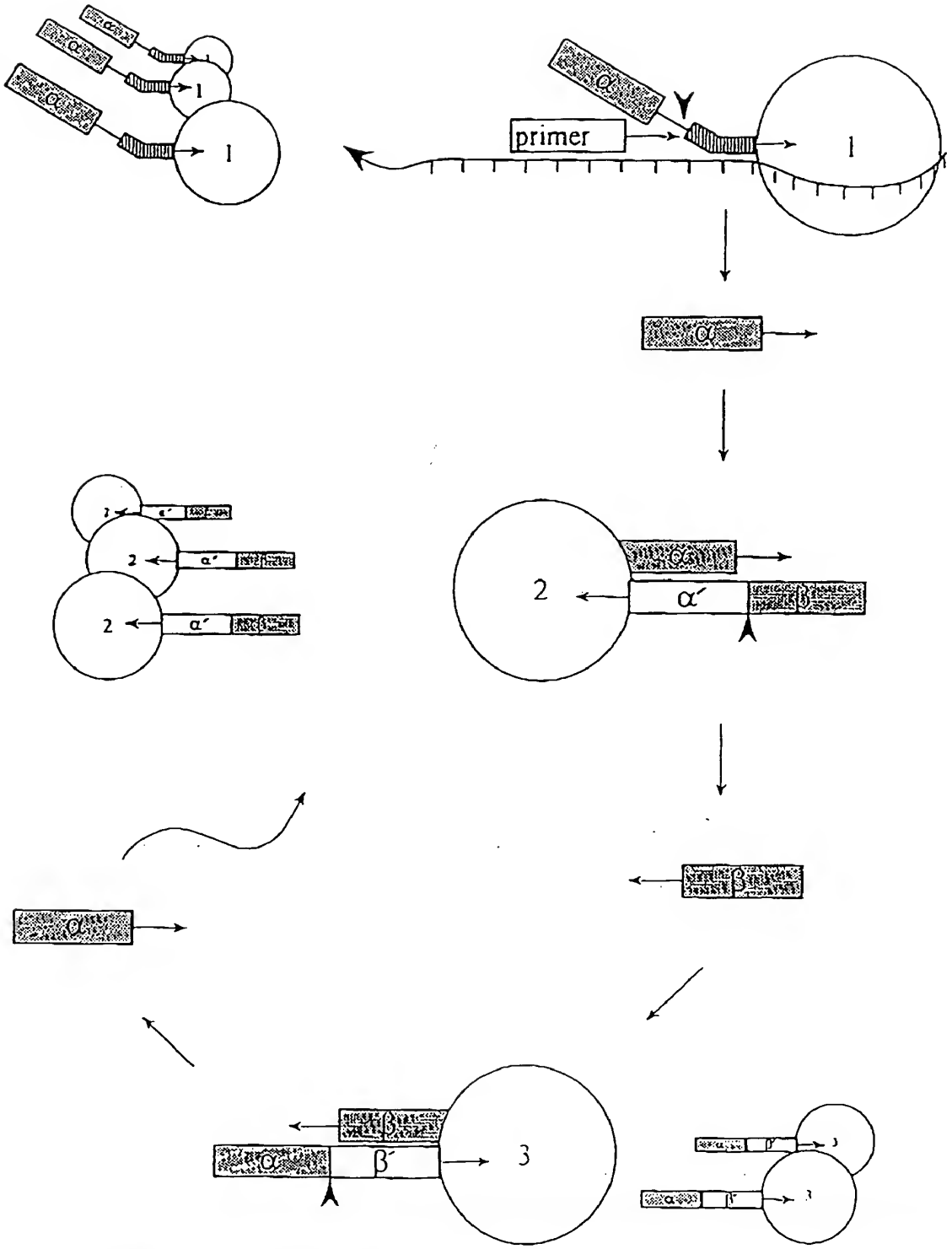
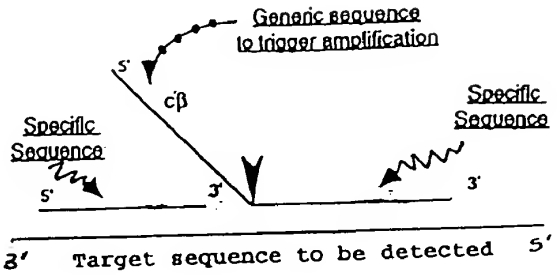
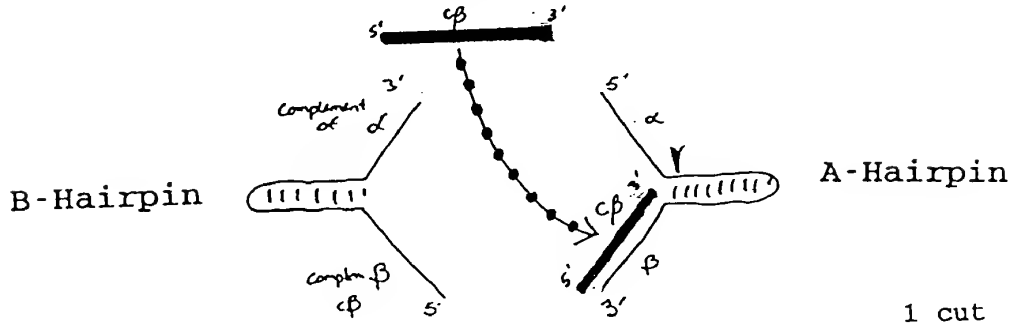


FIGURE 1 B

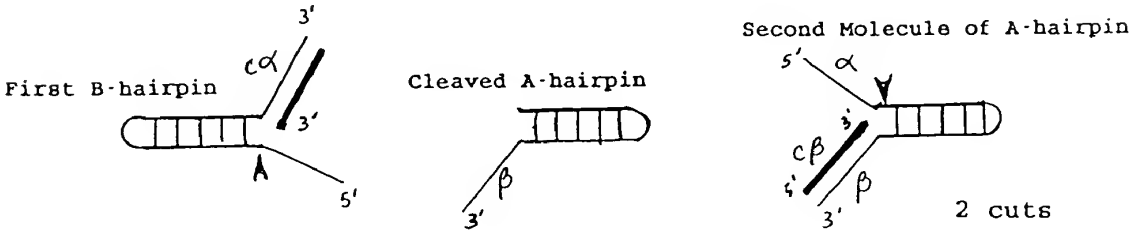
PART ONE: TRIGGER REACTION



PART TWO: DETECTION REACTION



Denature, anneal



Denature, anneal

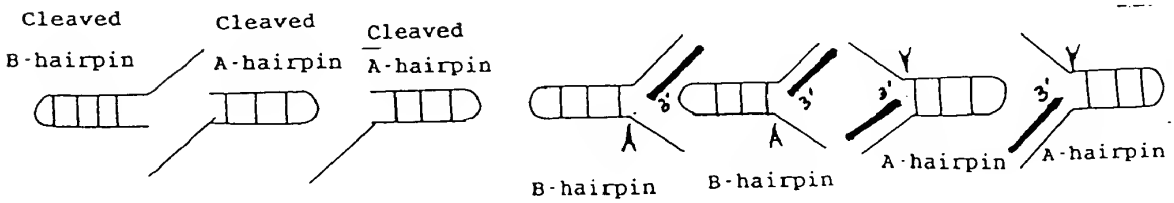


FIGURE 2

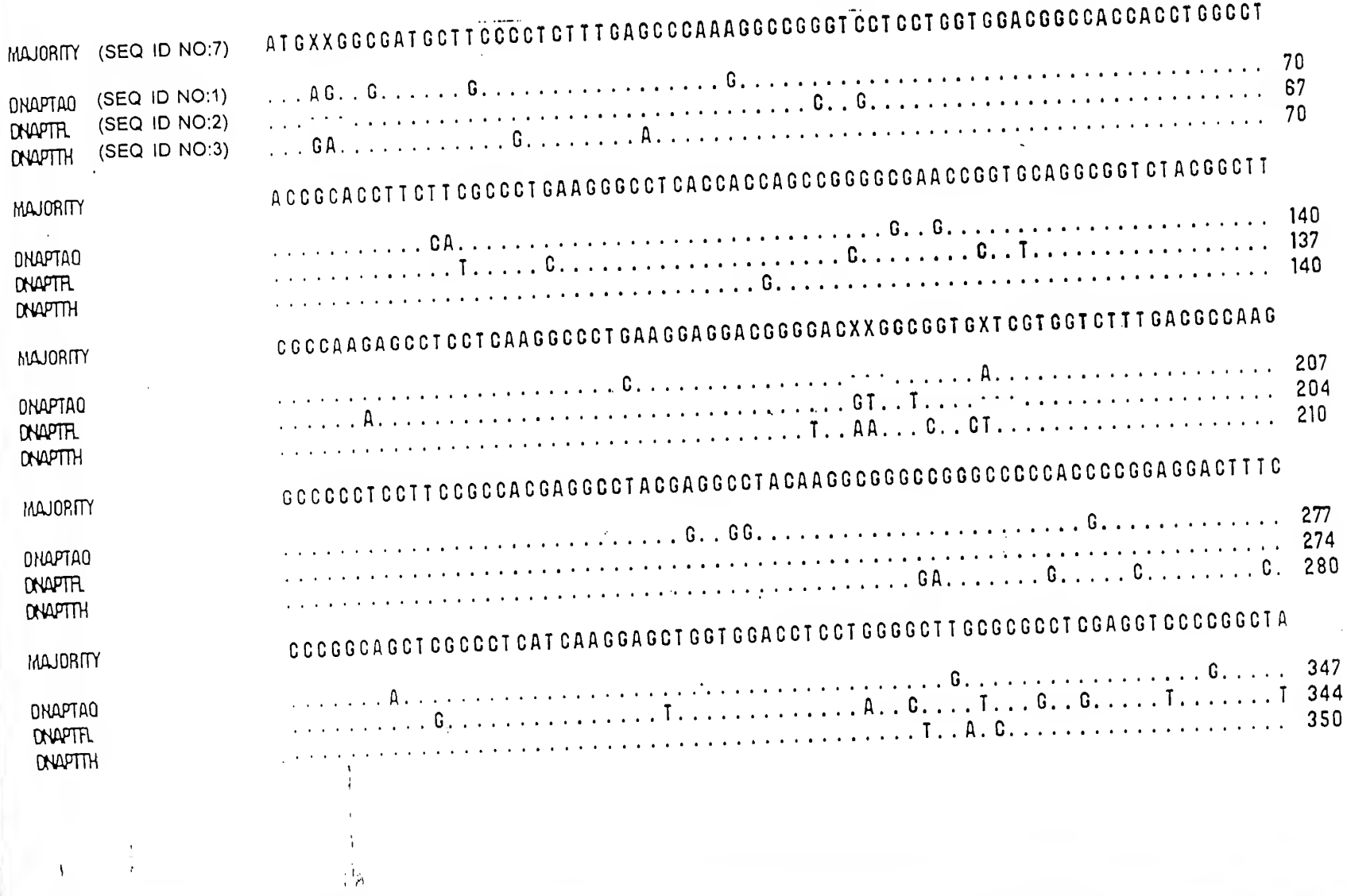
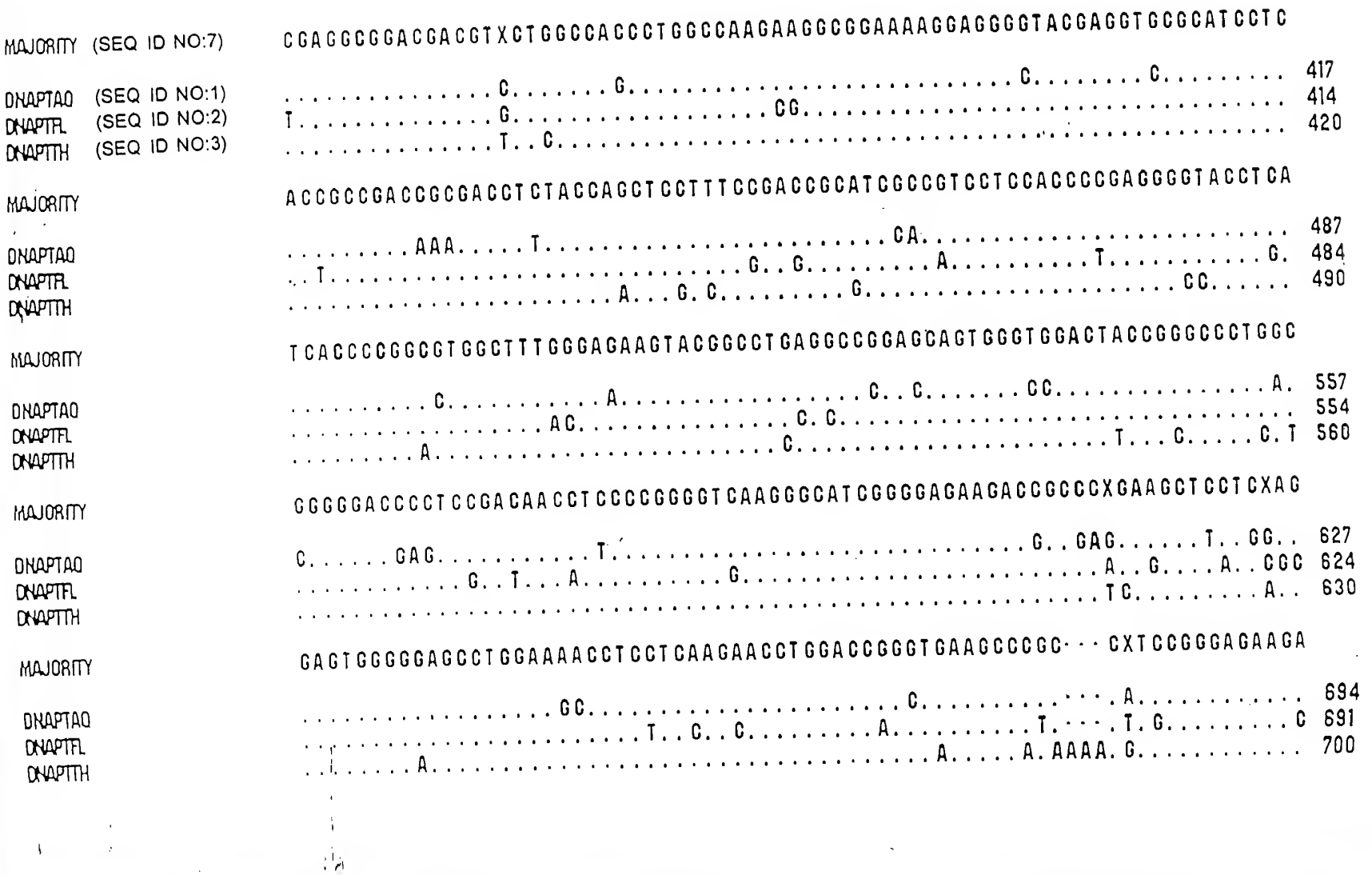


FIGURE 2 (cont'd)



08/520,946

FIGURE 2 (cont'd)

FIGURE 2 (cont'd)

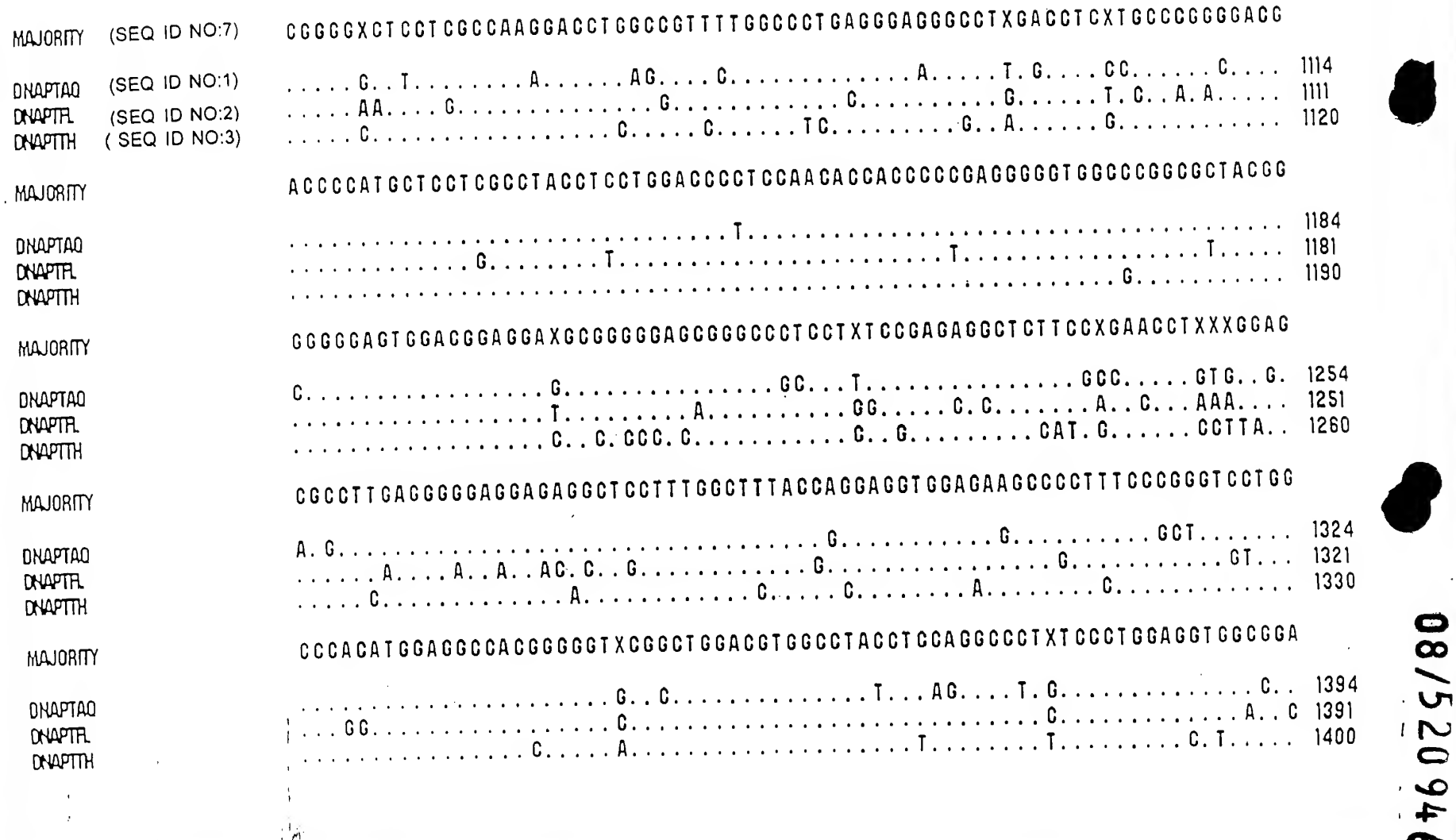


FIGURE 2 (cont'd)

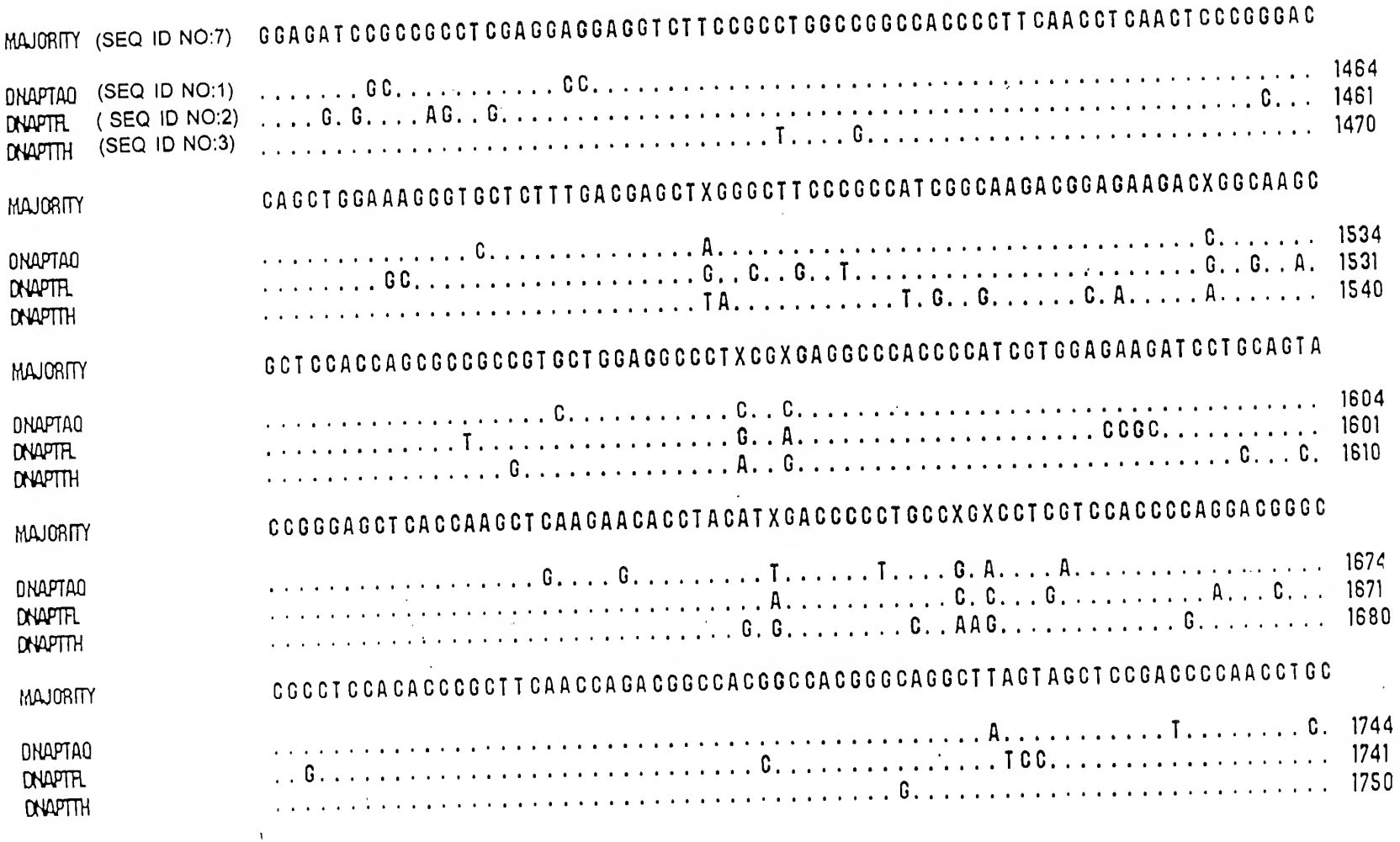


FIGURE 2 (cont'd)

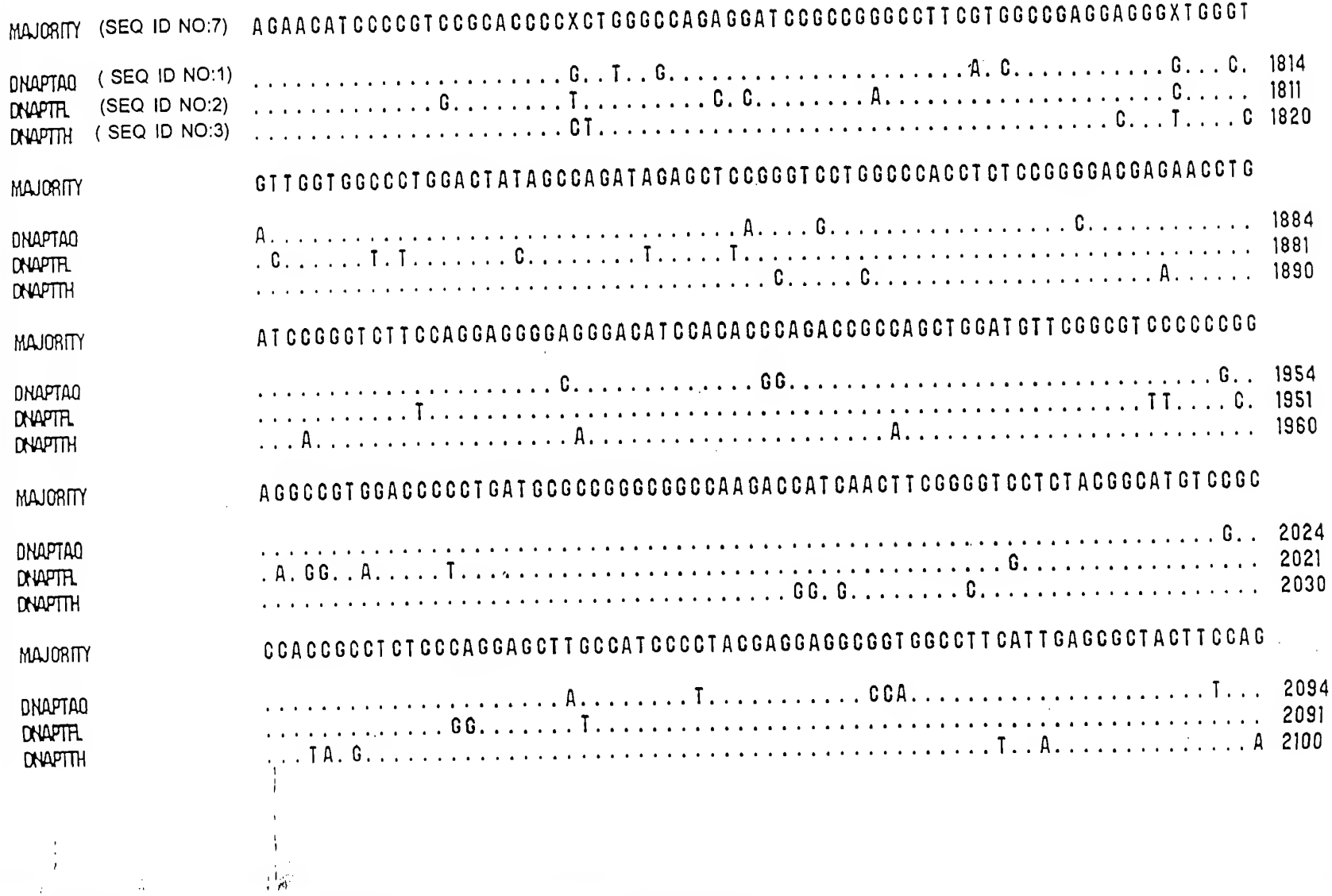


FIGURE 2 (cont'd)

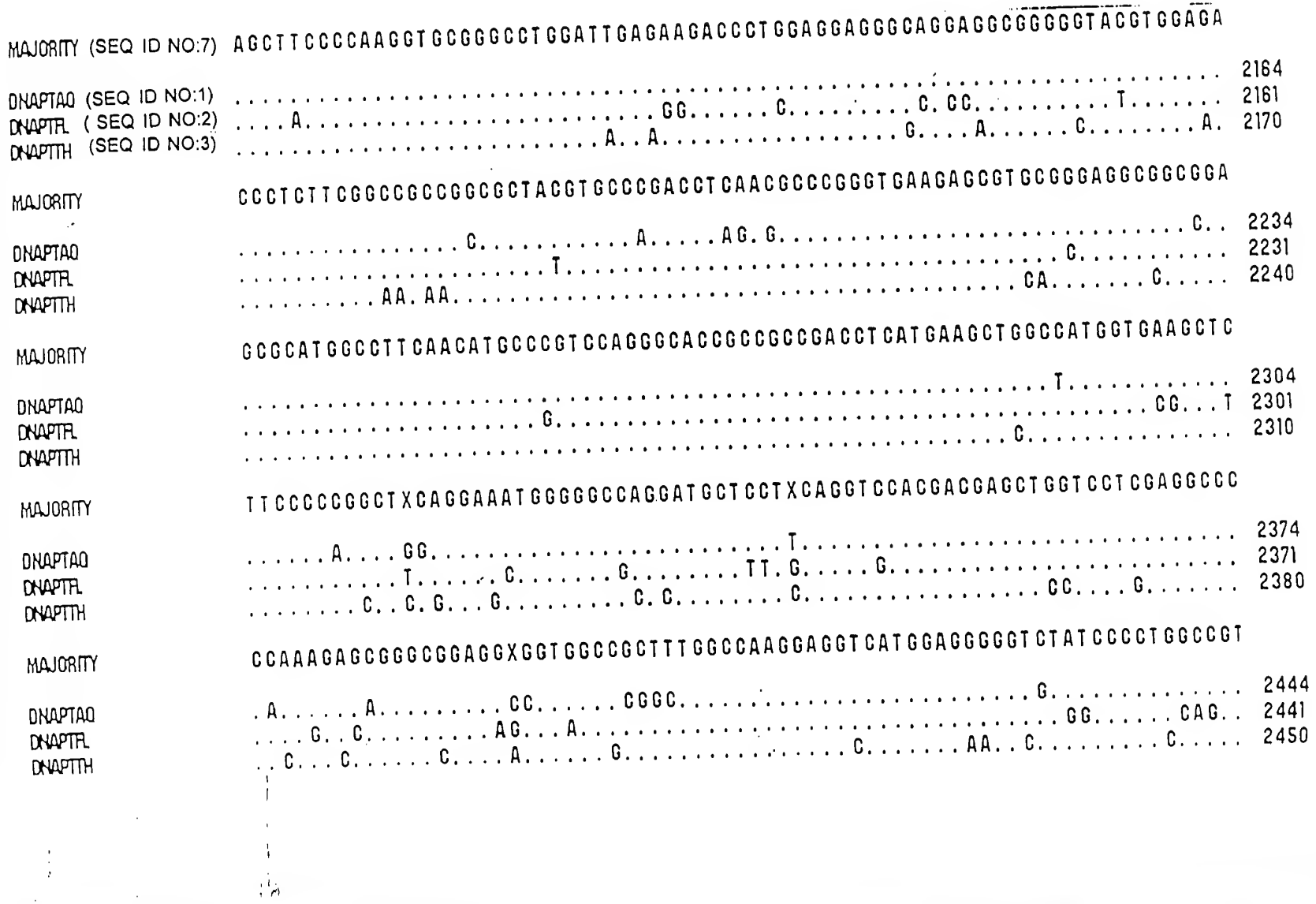


FIGURE 2 (cont'd)

```
MAJORITY   (SEQ ID NO:7)  GCCCCTGGAGGTGGAGGTGGGGATGGGGGAGGACTGGCTCTCCGCCAAGGAGTAG

DNAPTAQ    (SEQ ID NO:1)  .........................A.............................GA   2499
DNAPTFL    (SEQ ID NO:2)  .....................CC..............................     2496
DNAPTTH    (SEQ ID NO:3)  ...................................T..........GT...     2505
```

FIGURE 3

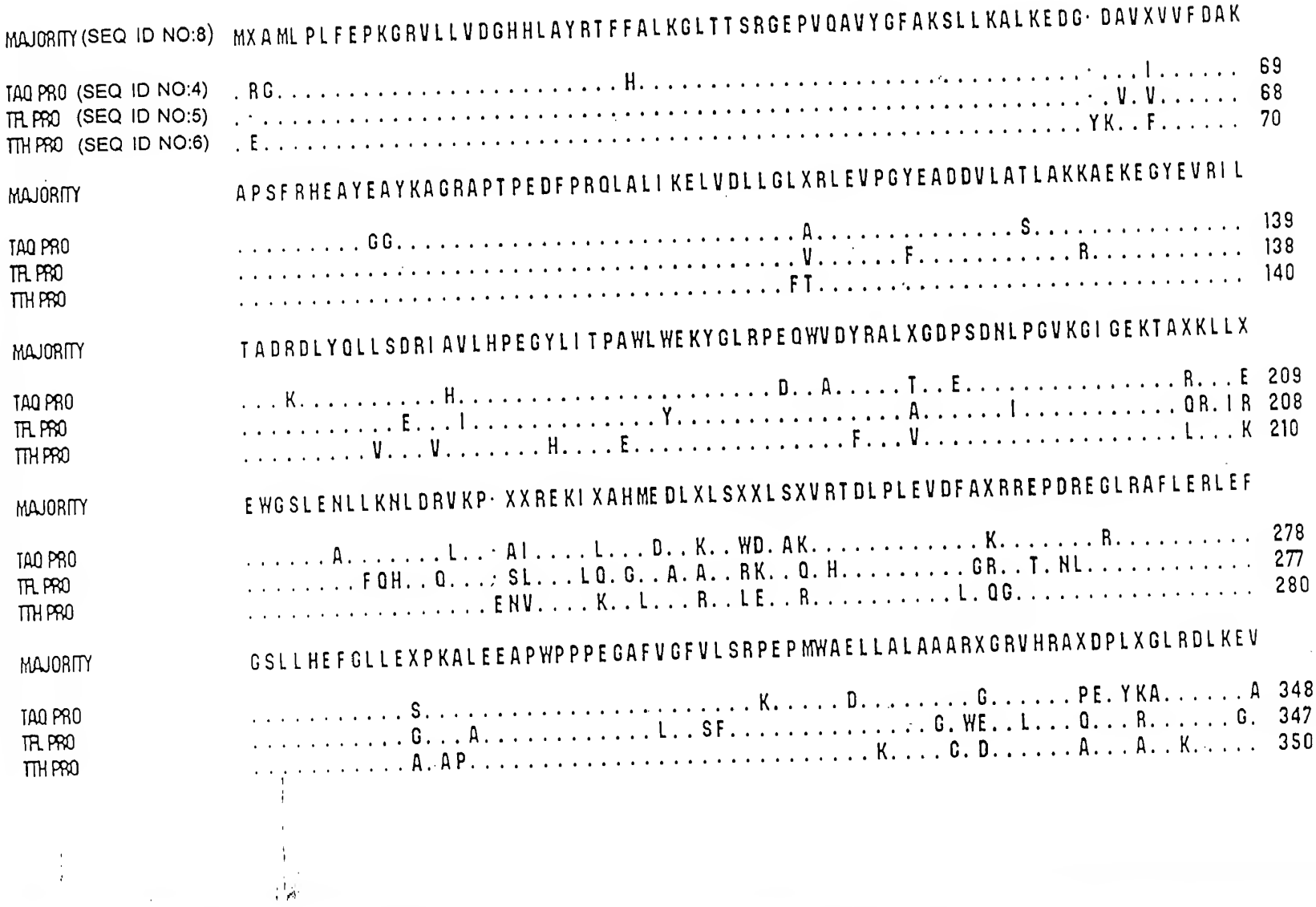


FIGURE 3 (cont'd)

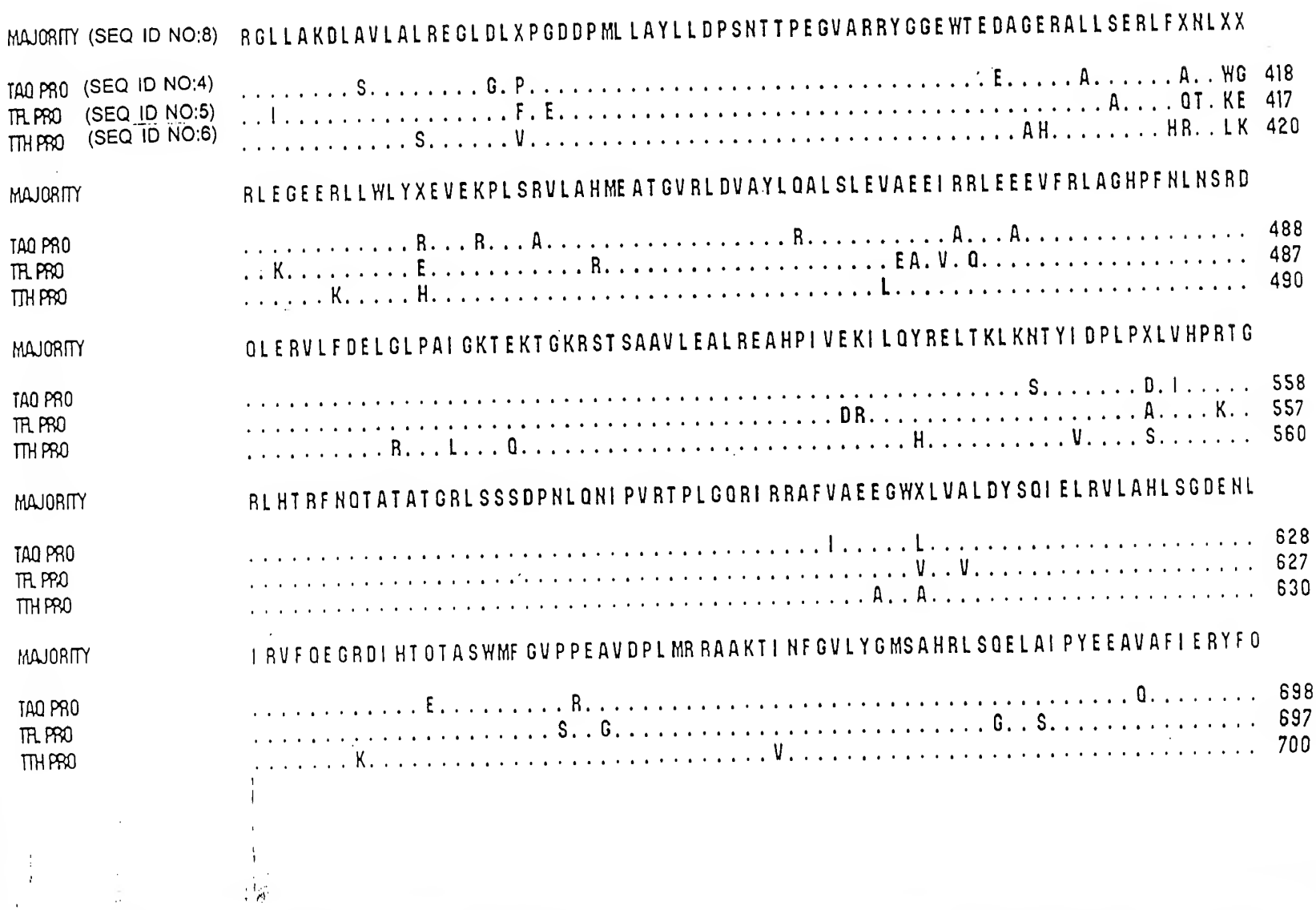


FIGURE 3 (cont'd)

```
MAJORITY (SEQ ID NO:8)  SFPKVRAWIEKTLEEGRRRGYVETLFGRRRYVPDLNARVKSVREAAERMAFNMPVQGTAADLMKLAMVKL

TAQ PRO  (SEQ ID NO:4)  ..........................................E.....................................  768
TFL PRO  (SEQ ID NO:5)  .Y.......G.....................................................................R.  767
TTH PRO  (SEQ ID NO:6)  ................K.....................................................................  770

MAJORITY               FPRLXEMGARMLLQVHDELVLEAPKXRAEXVAALAKEVMEGVYPLAVPLEVEVGXGEDWLSAKEX

TAQ PRO                ....E..................E...A..R.....................I.........  833
TFL PRO                ....Q.L..................D...R.............W..Q.........L.........  831
TTH PRO                ....R..............L....QA...E.......A..KA.............M........G  835
```

FIGURE 4

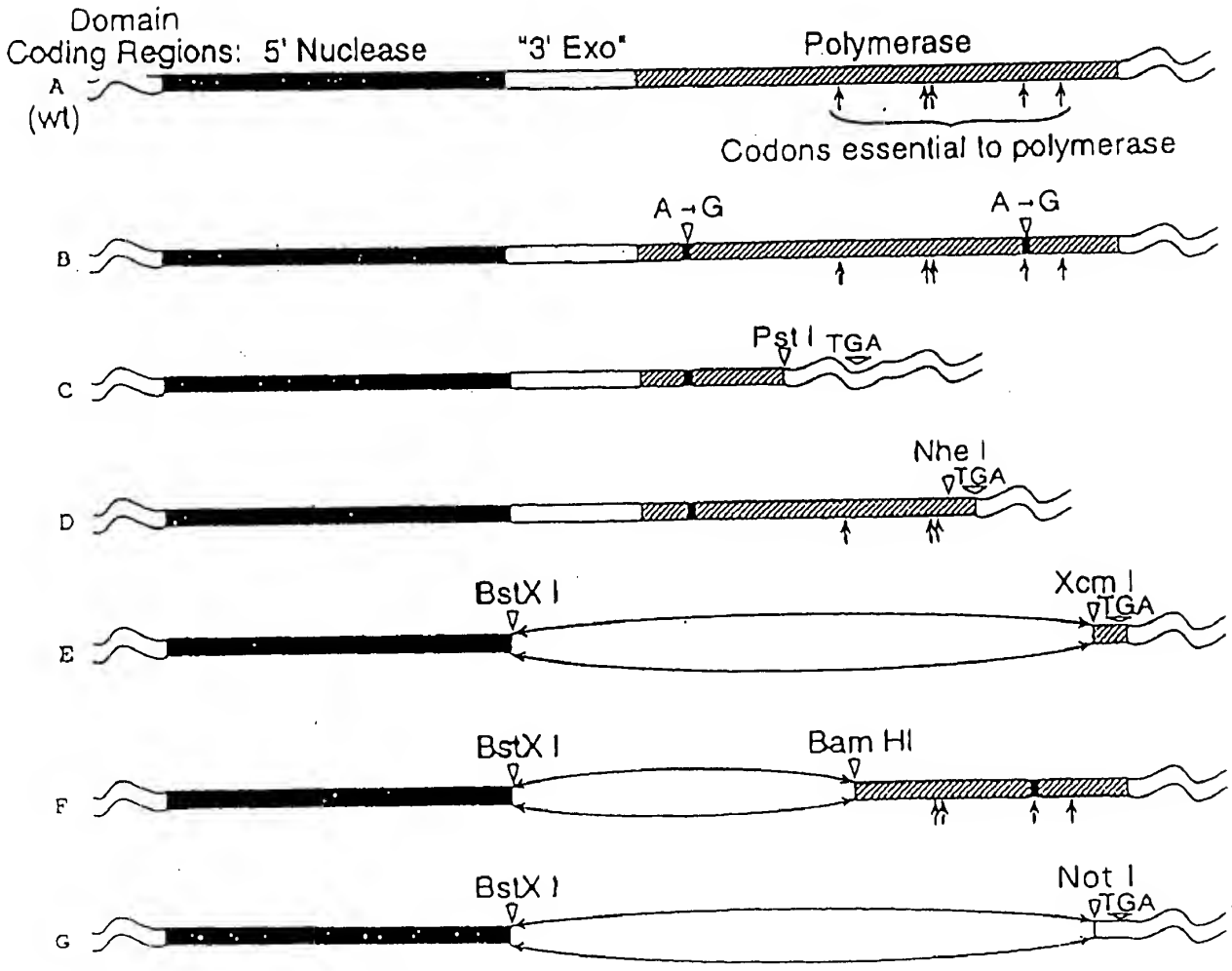


FIGURE 5

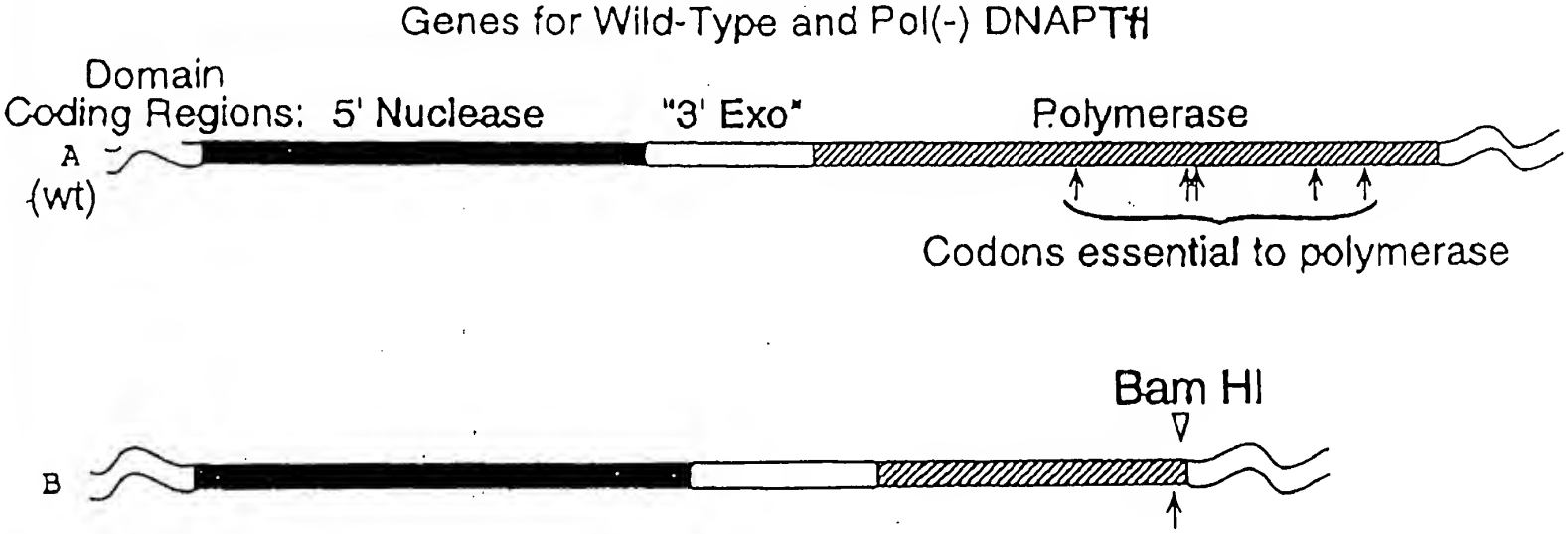


FIGURE 6

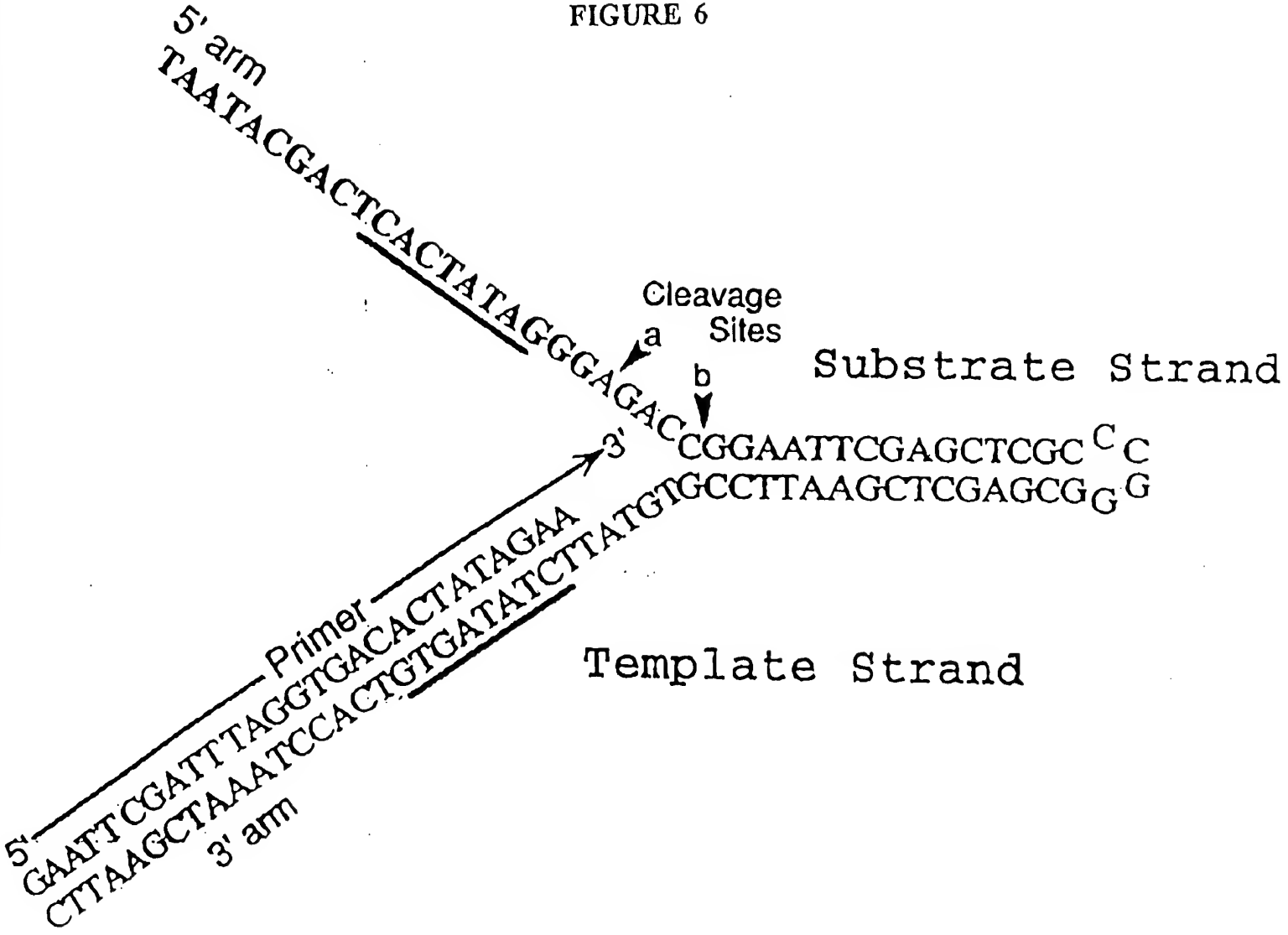


FIGURE 7

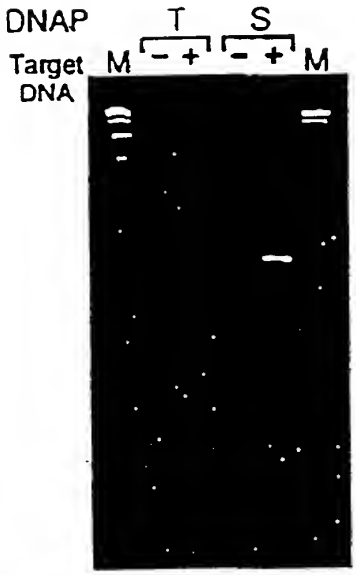


FIGURE 8

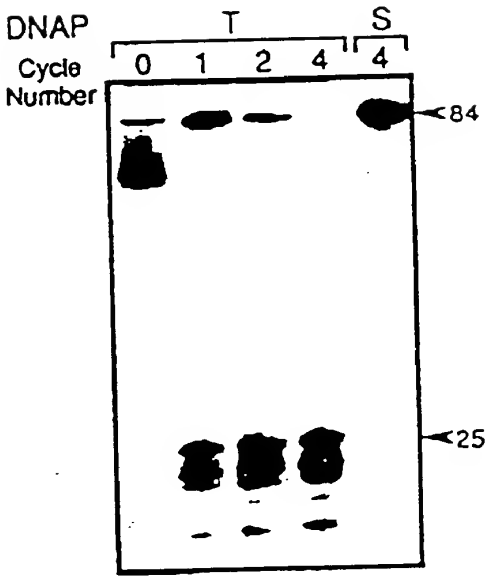


FIGURE 9

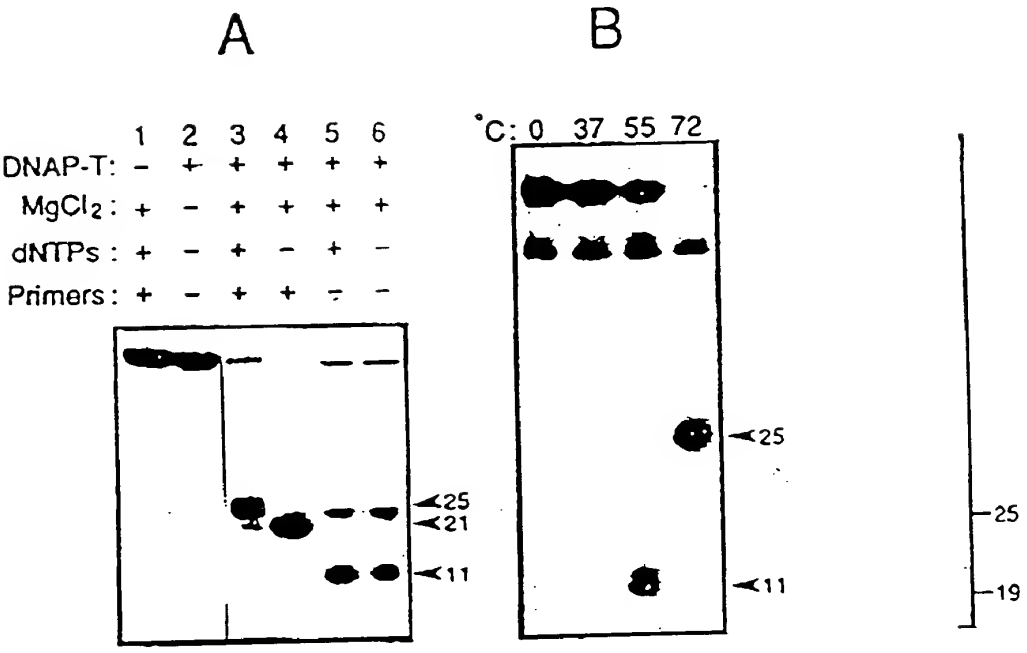

A
B
1 2 3 4 5 6
DNAP-T: - + + + + +
MgCl2: + - + + + +
dNTPs: + - + - + -
Primers: + - + + + -
25
21
11
°C: 0 37 55 72
25
11
25
19

FIGURE 10

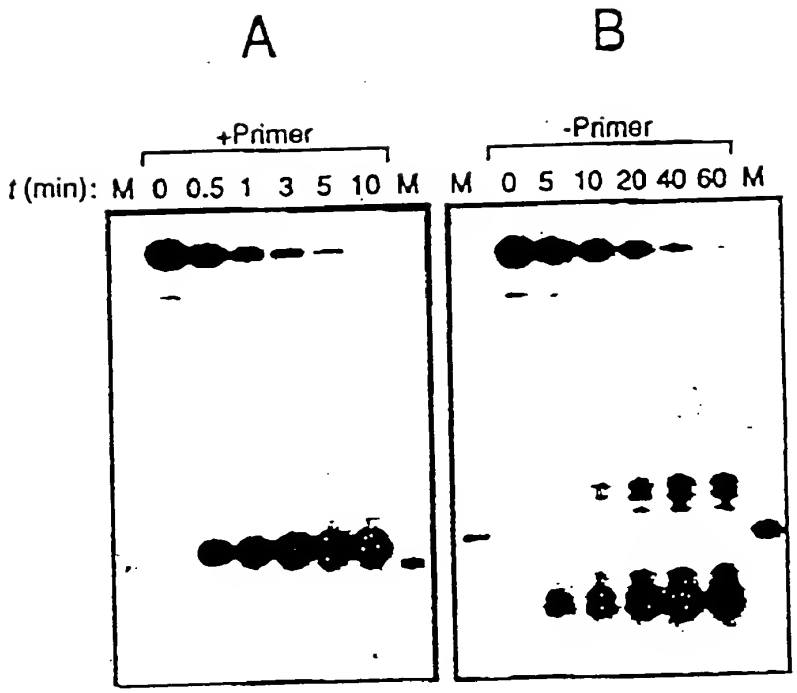


FIGURE 11

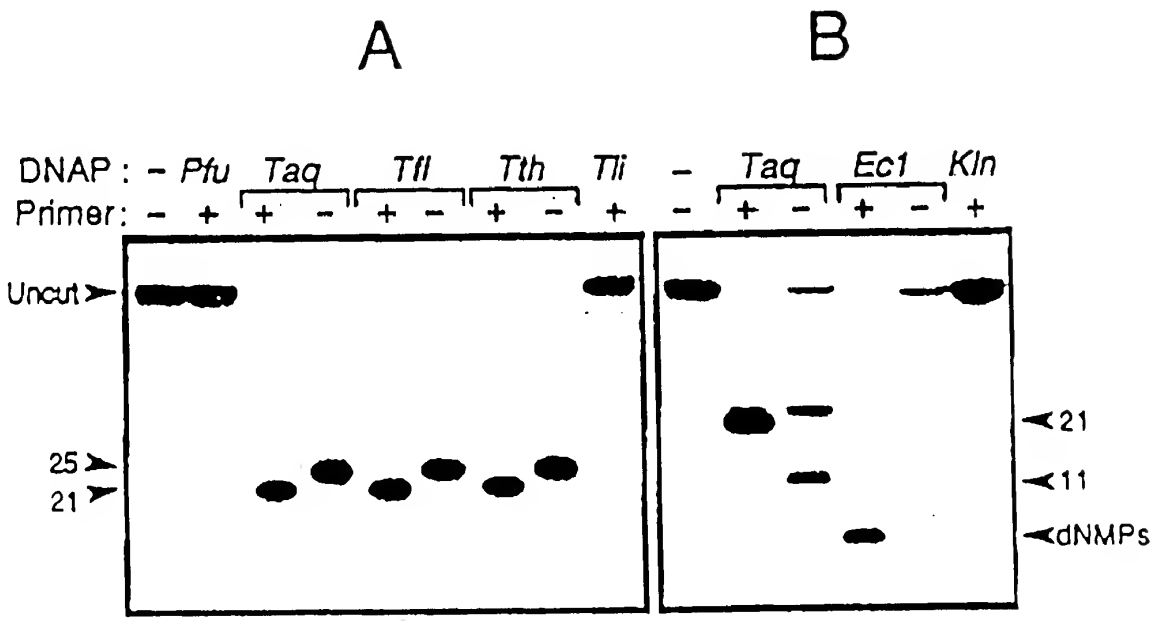


FIGURE 12

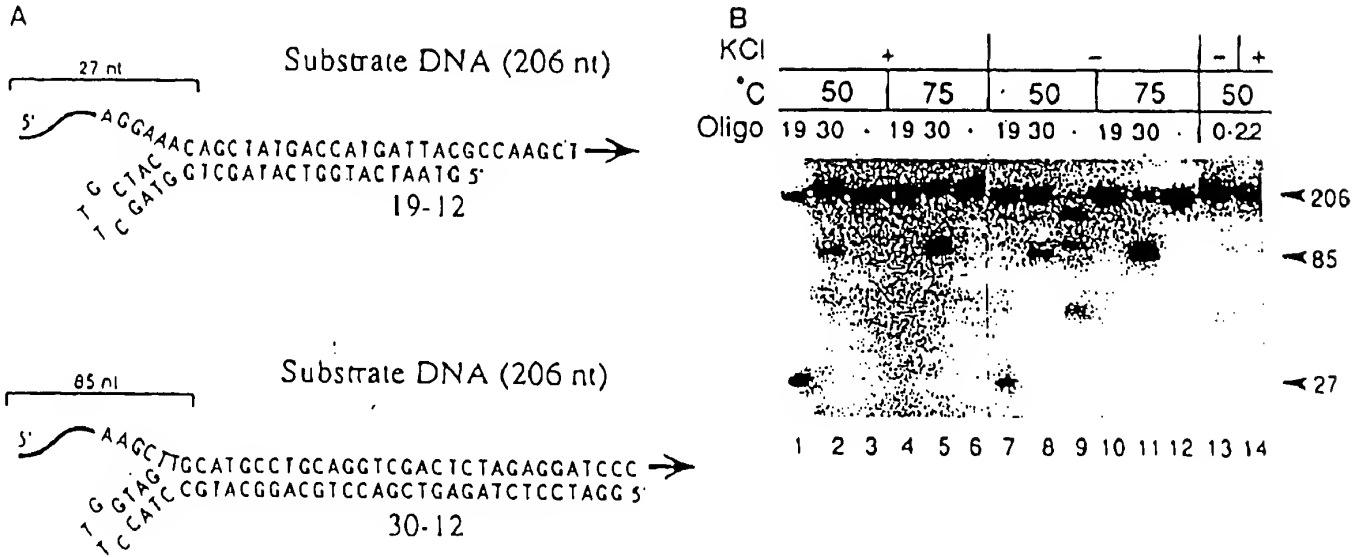


FIGURE 13

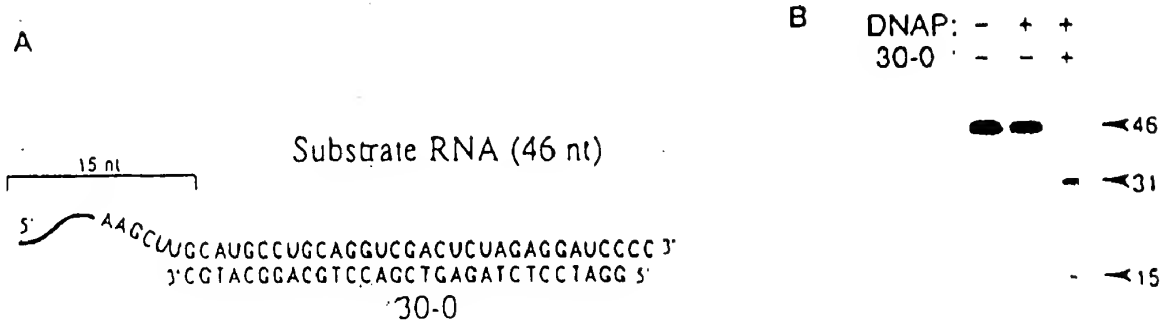
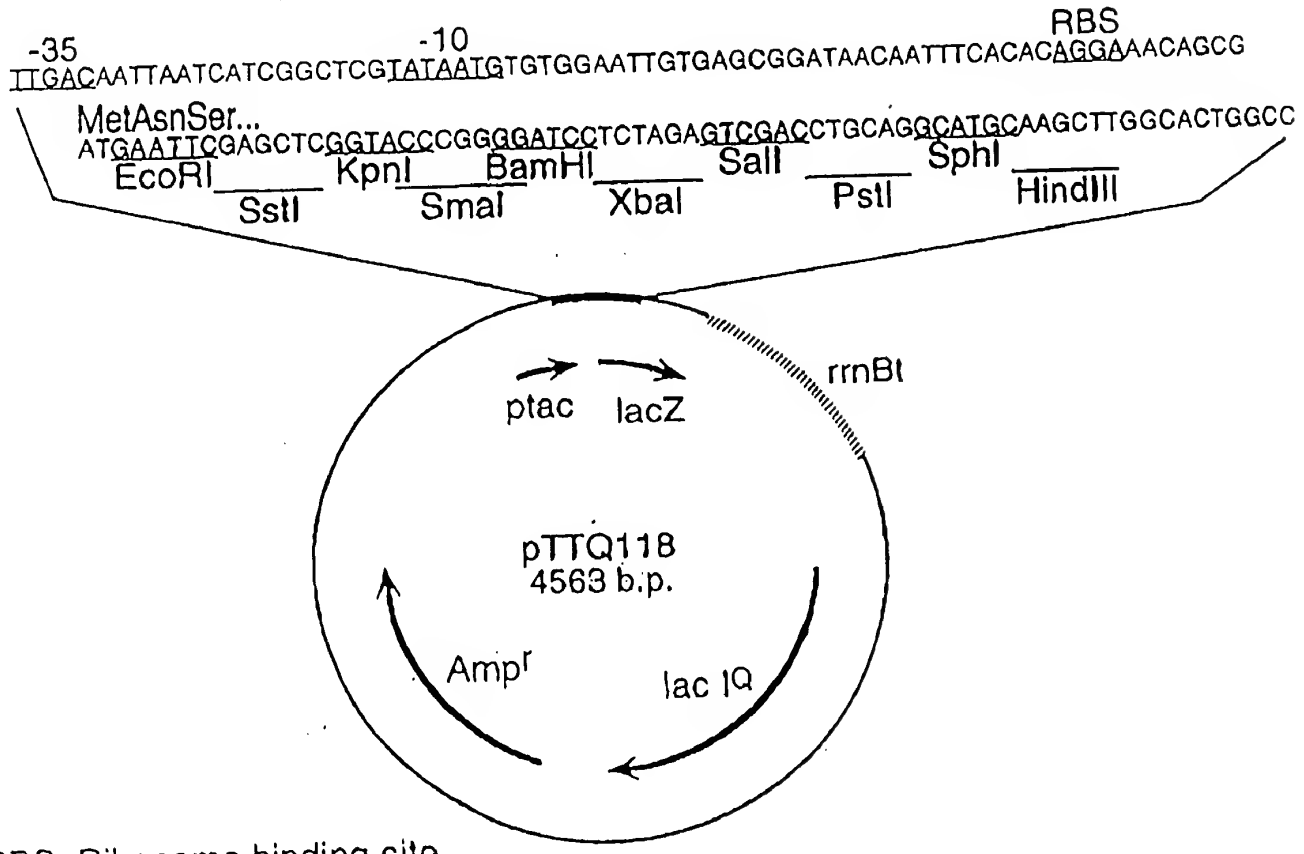


FIGURE 14



RBS: Ribosome binding site

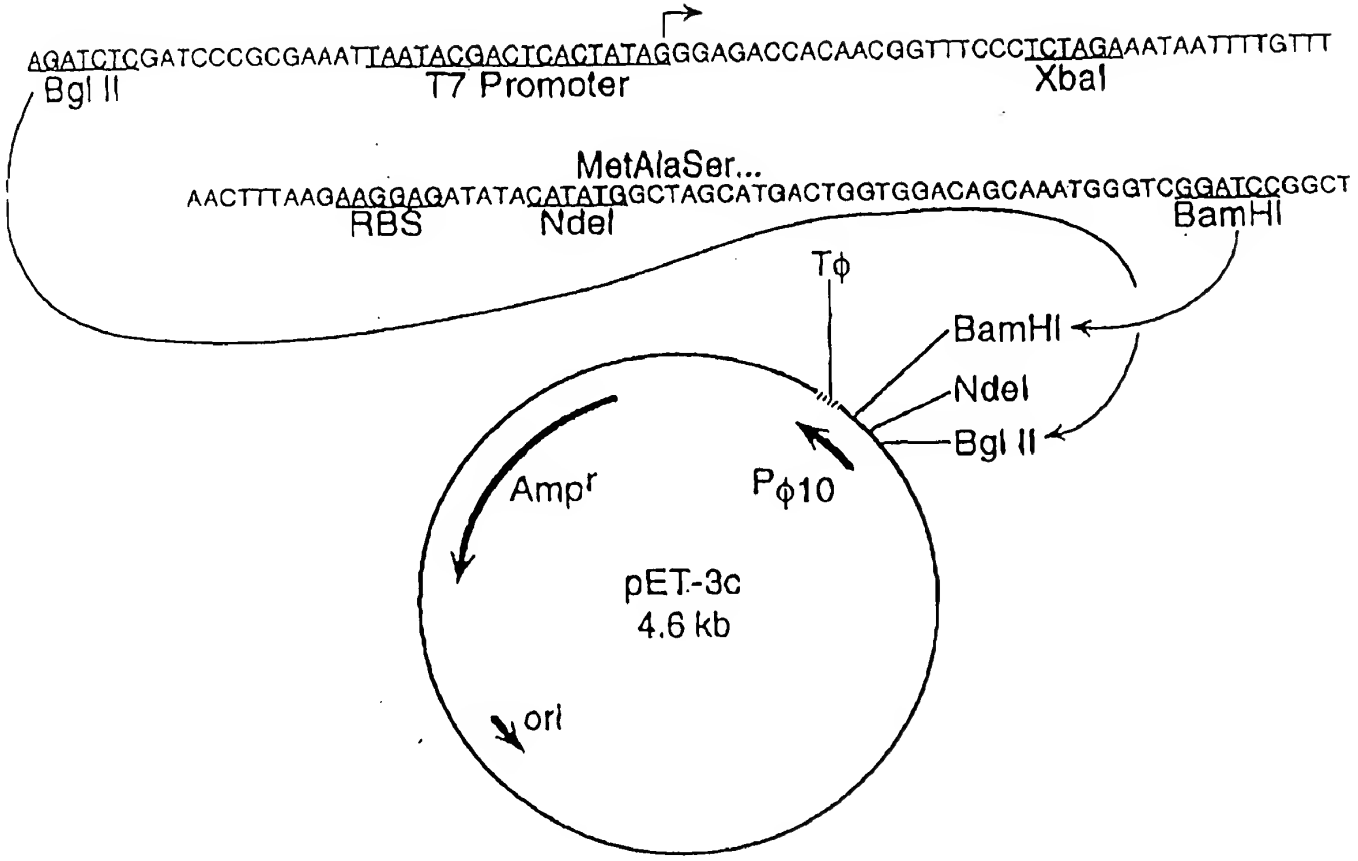
ptac: Synthetic tac promoter

lac I^Q: Lac repressor gene

lacZ: Beta-galactosidase alpha fragment

rrnBt: E. coli rrnB transcription terminator

FIGURE 15



$P_{\phi 10}$: Bacteriophage T7 ϕ10 promoter

RBS: Ribosome binding site

Tϕ: T7 ϕ Terminator

FIGURE 16

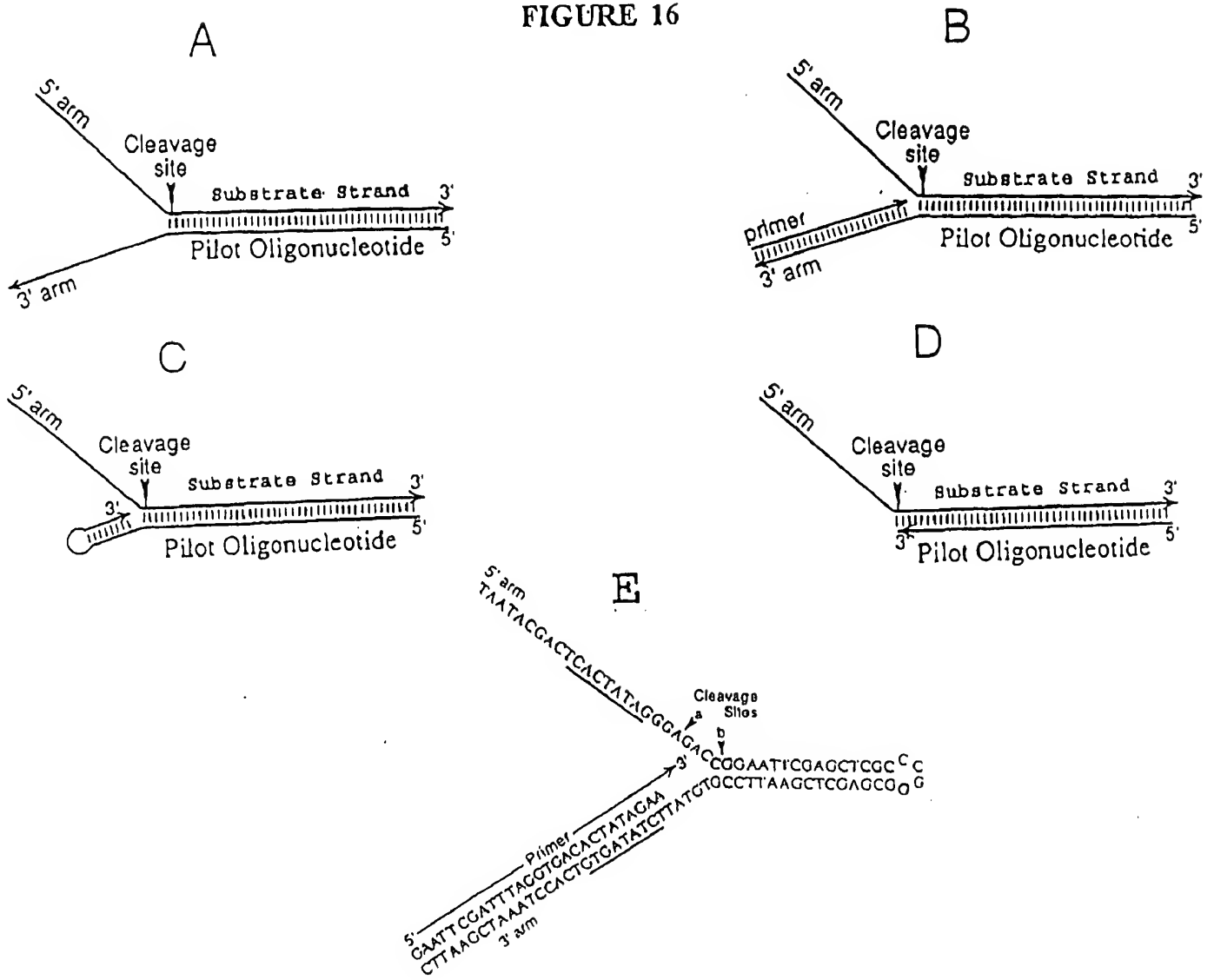


FIGURE 17

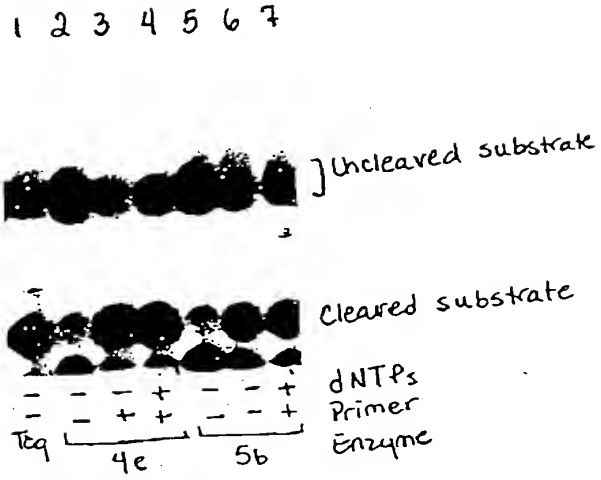

1 2 3 4 5 6 7
Uncleaved substrate
Cleaved substrate
dNTPs
Primer
Enzyme
Taq
4e
5b

FIGURE 18

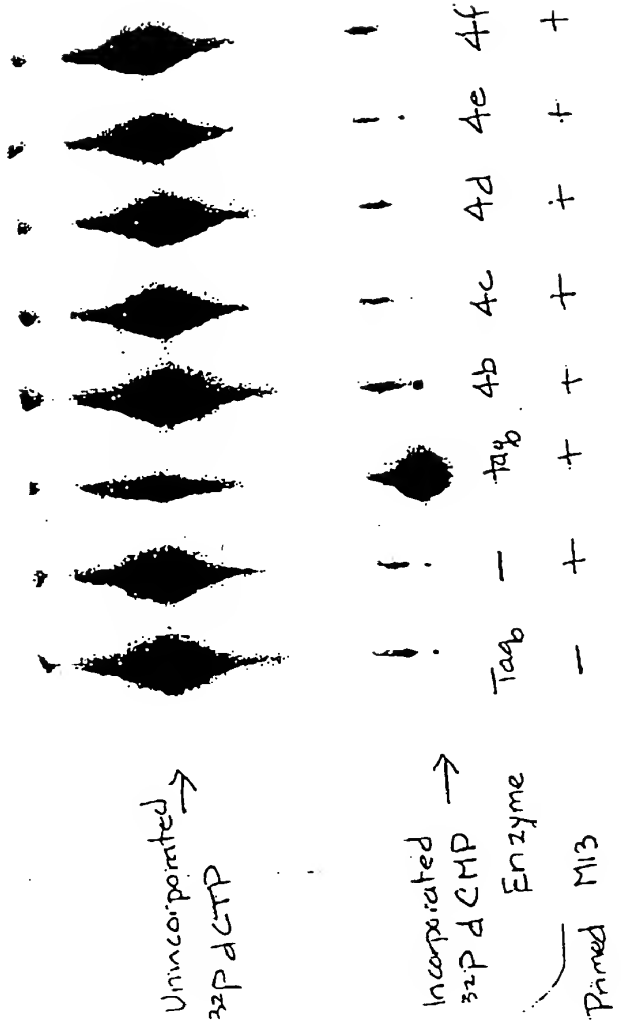
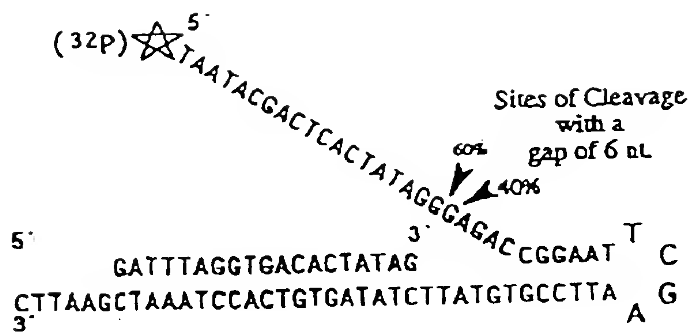

Unincorporated
32P dCTP
Incorporated
32P dCMP
Enzyme
Taq
Taq
Primed M13
4b
4c
4d
4e
4f

FIGURE 19

A


(32P)
5'
TAATACGACTCACTATAGGGAGAC
Sites of Cleavage with a gap of 6 nt.
60%
40%
3'
5'
GATTTAGGTGACACTATAG
CCGGAAT
T
C
CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTTA
A
G
3'

B

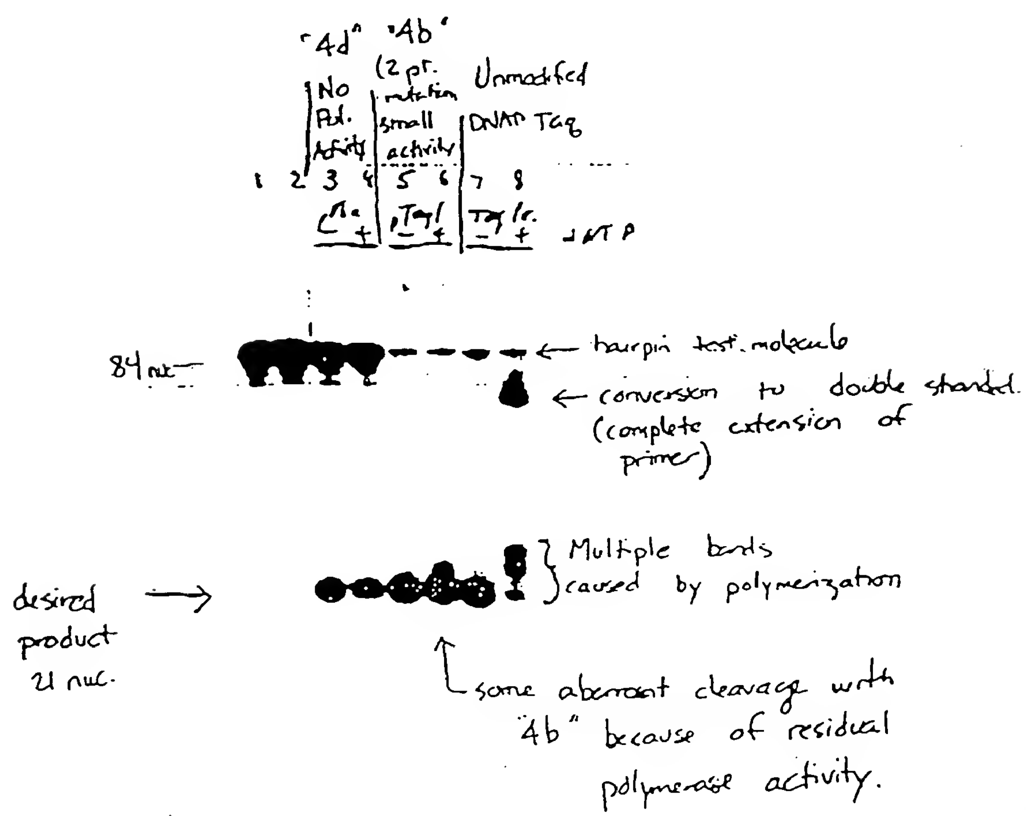
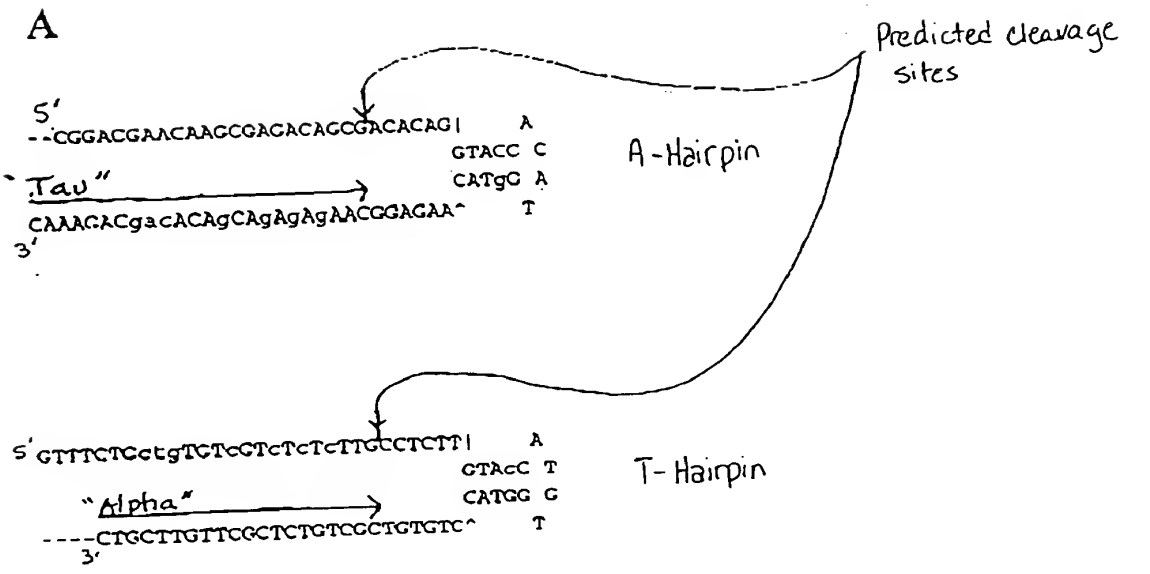
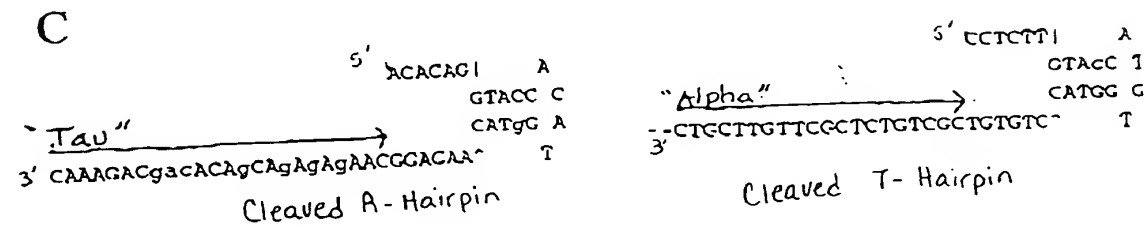

"4d"
"4b"
No Pol. Activity
(2 pt. mutation small activity
Unmodified DNAP Taq
1 2 3 4 5 6 7 8
dNTP
84 nt
hairpin test molecule
conversion to double standard. (complete extension of primer)
Multiple bands caused by polymerization
desired product 21 nuc.
some aberrant cleavage with "4b" because of residual polymerase activity.

FIGURE 20



B Sequence of alpha primer:

5' GAC GAA.CAA.CCG AGA CAG CG 3'



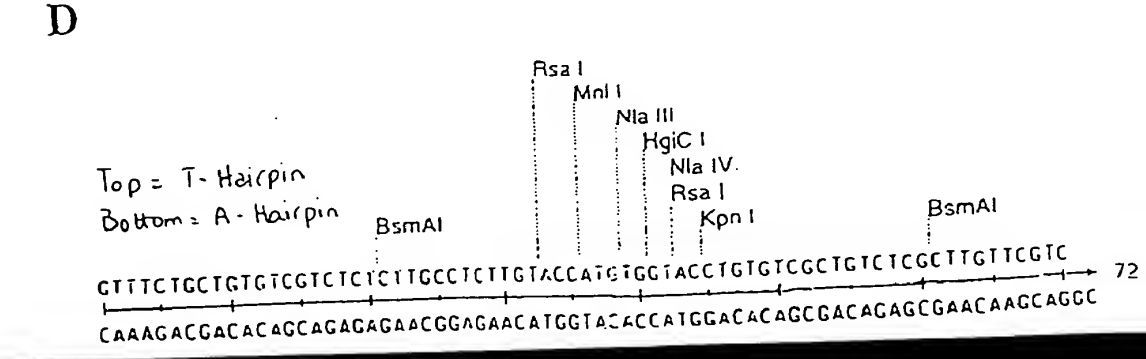


FIGURE 21

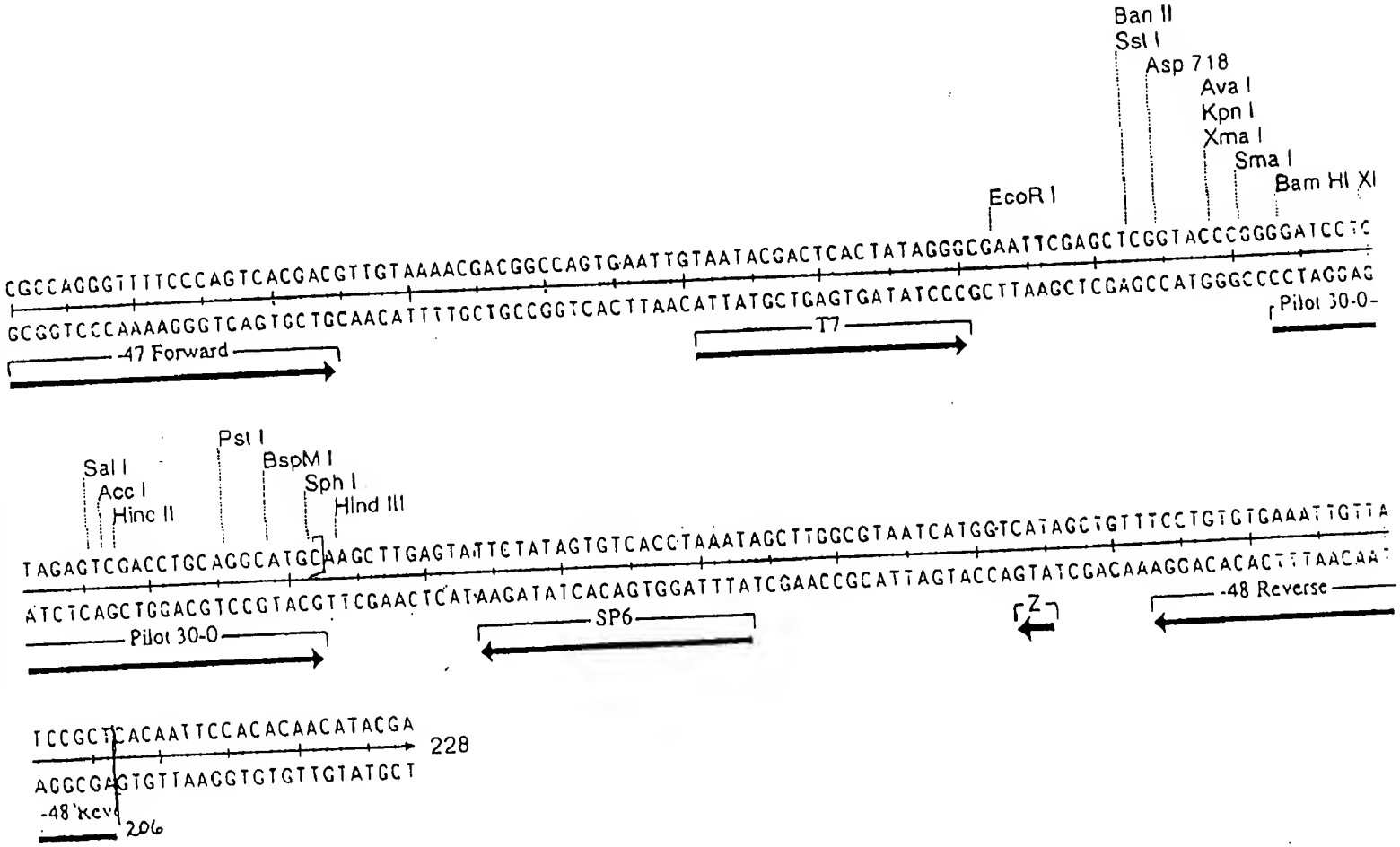


FIGURE 22A

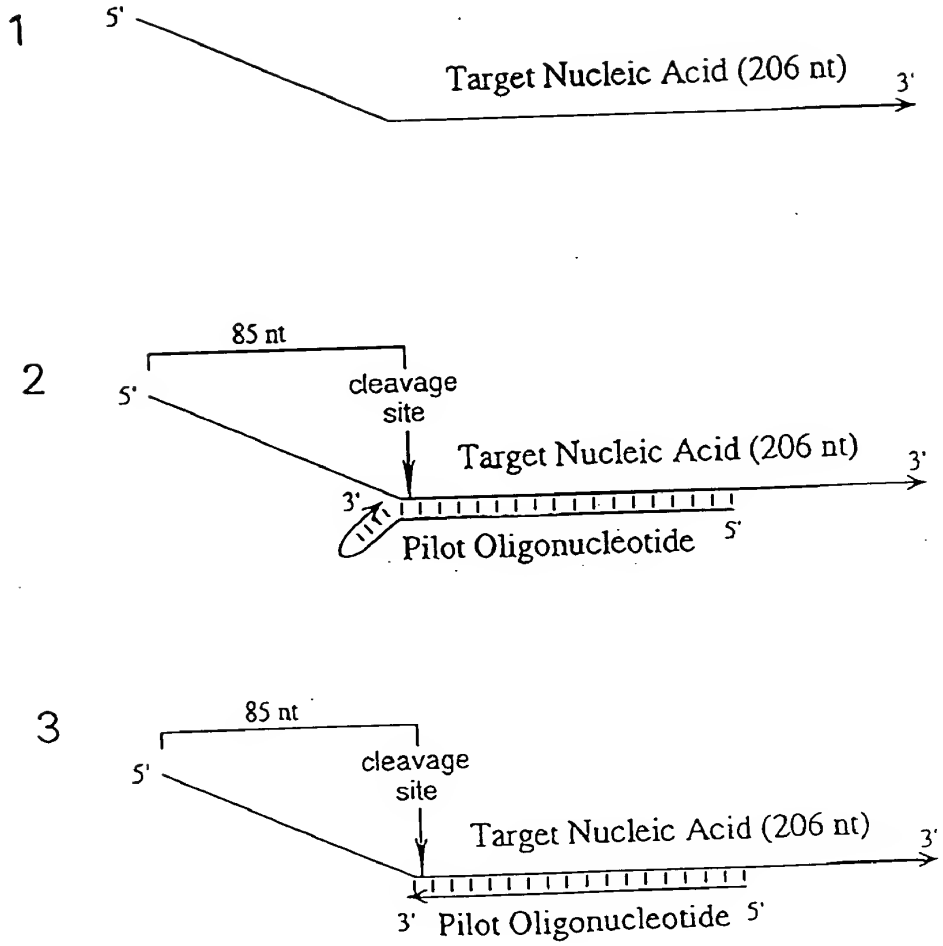

1
5'
Target Nucleic Acid (206 nt)
3'
2
85 nt
5'
cleavage site
Target Nucleic Acid (206 nt)
3'
3'
Pilot Oligonucleotide
5'
3
85 nt
5'
cleavage site
Target Nucleic Acid (206 nt)
3'
3'
Pilot Oligonucleotide
5'

FIGURE 22B

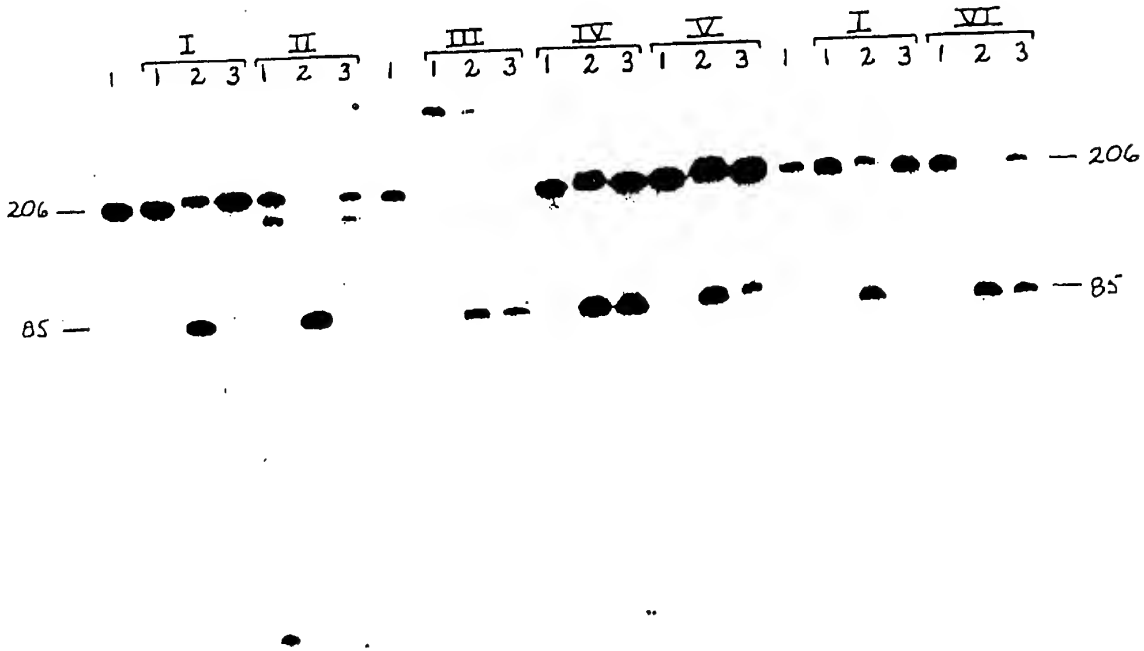


FIGURE 23

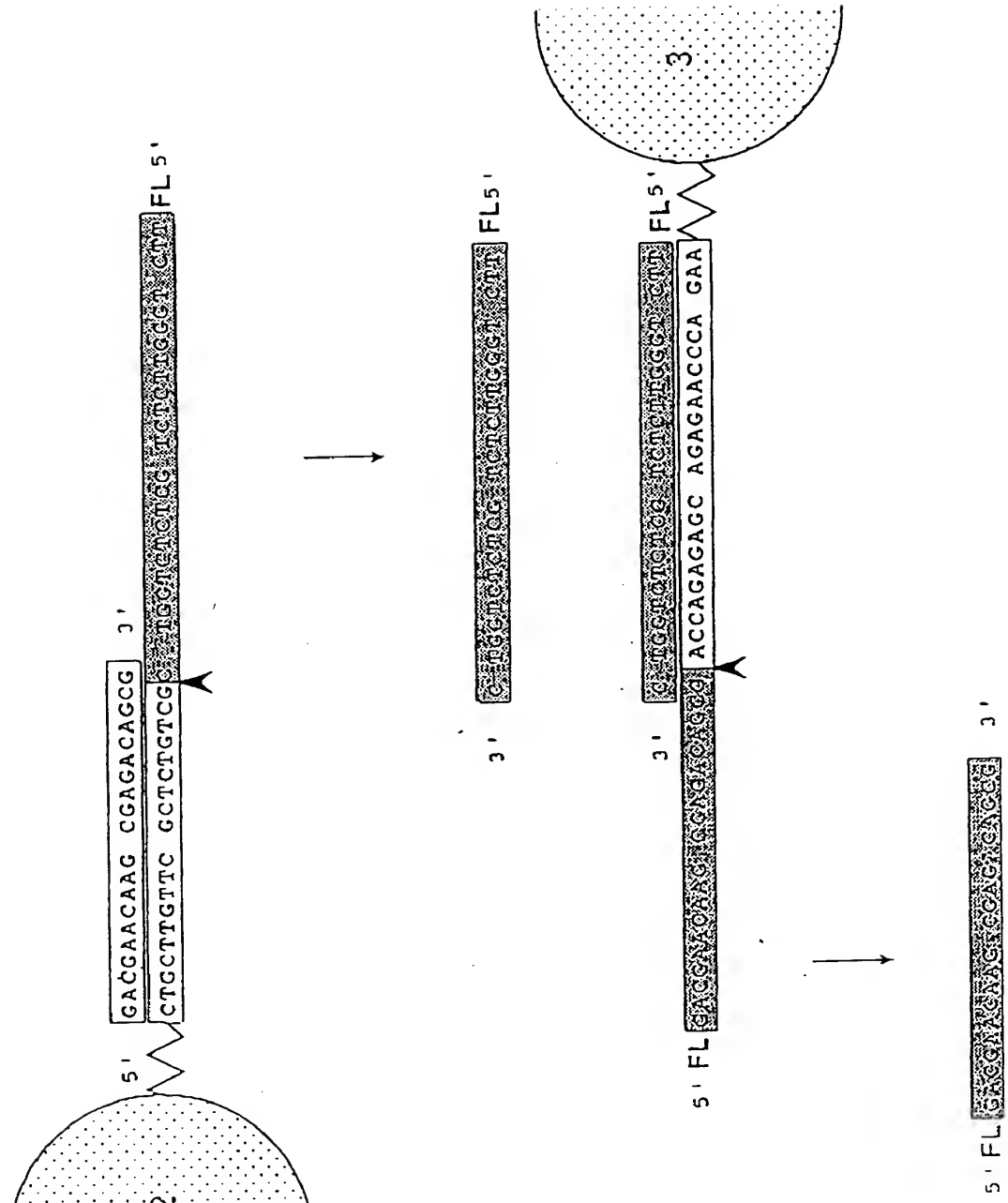

5'
GACGAACAAG CGAGACAGCG
3'
CTGCTTGTTC GCTCTGTCGC
FL 5'
2
3'
FL 5'
3
FL 5'
ACCAGAGAGC AGAGAACCCA GAA
3'
5' FL
5' FL
3'

FIGURE 24

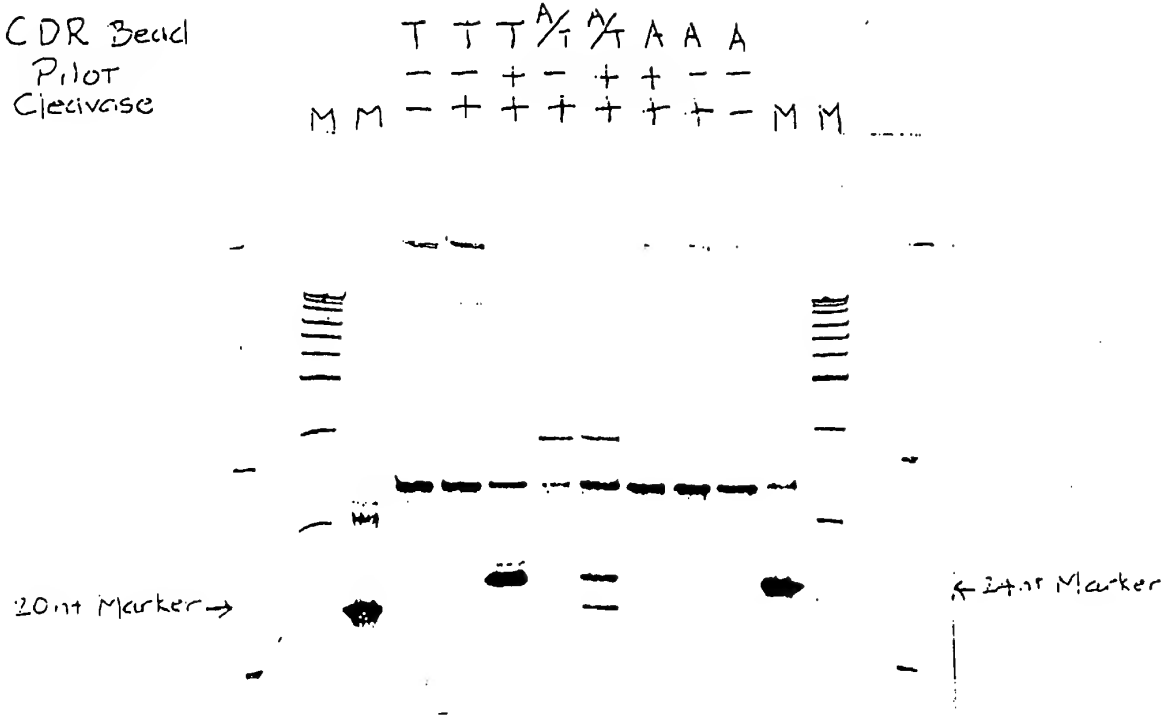


FIGURE 25

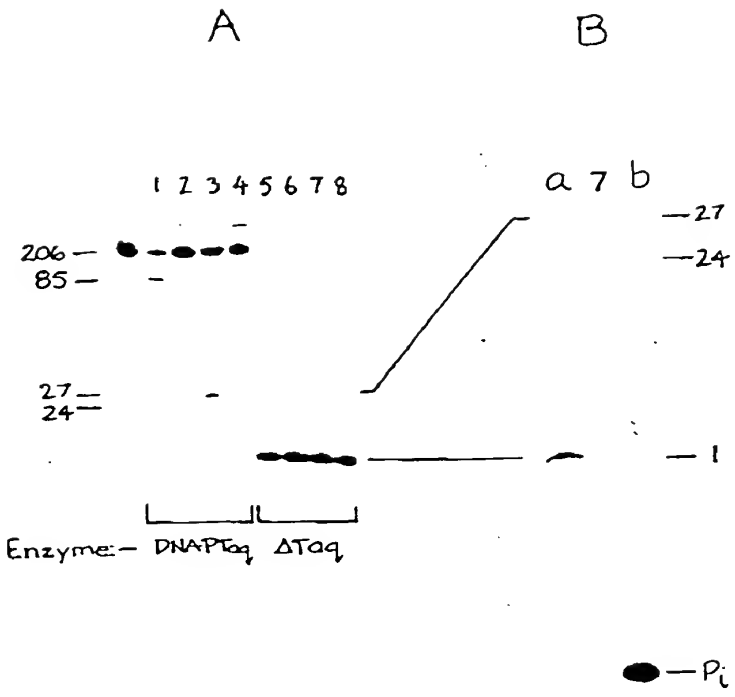
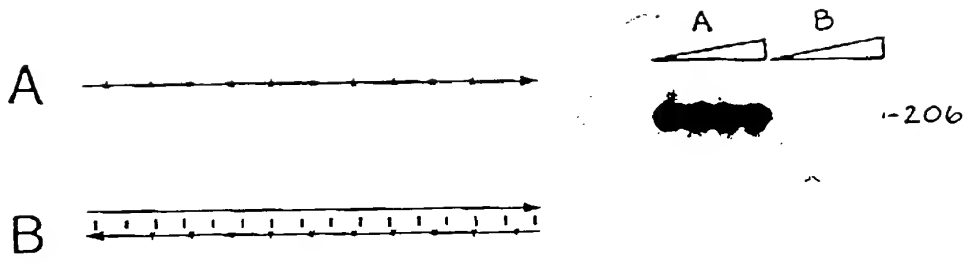


FIGURE 26



* = ^{32}P

FIGURE 27

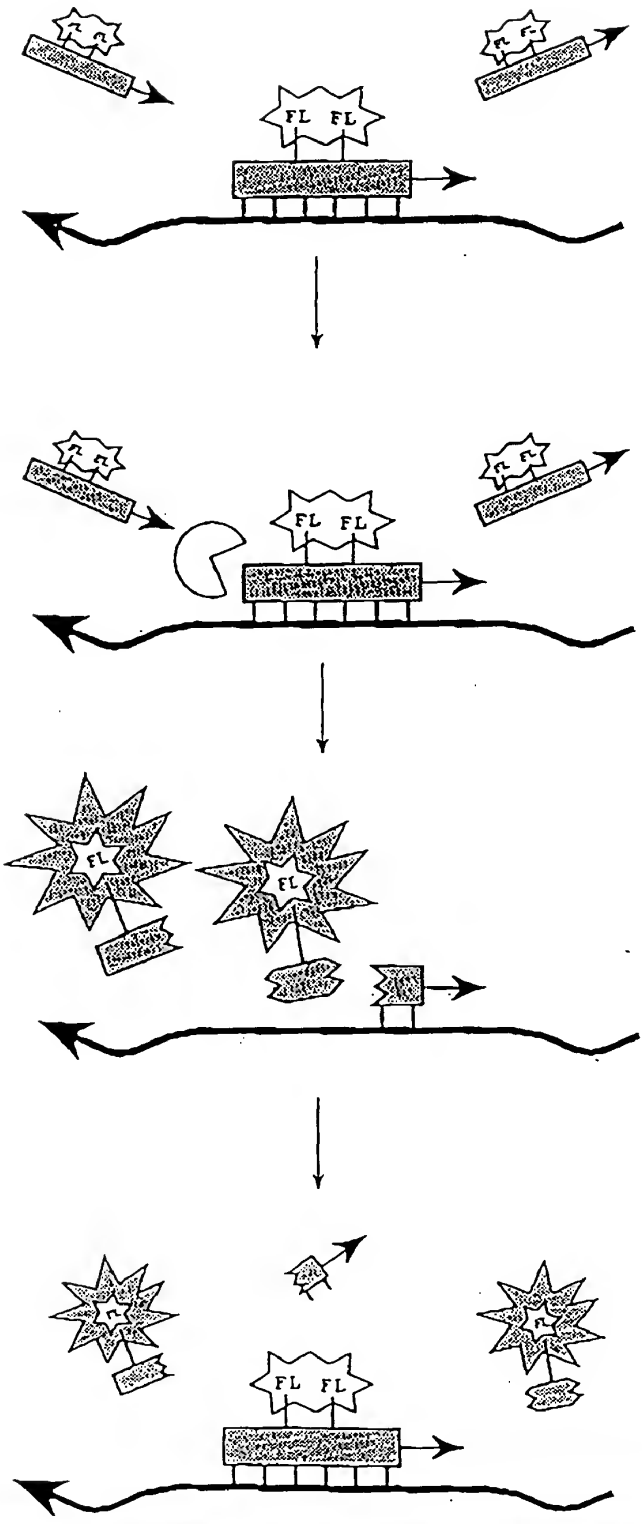
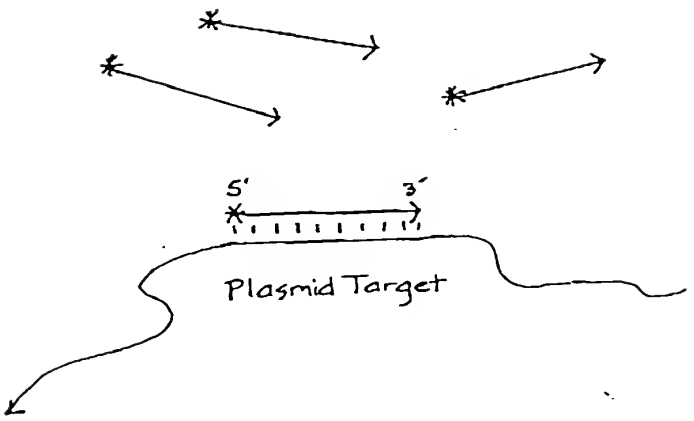


FIGURE 28A



* = ^{32}P 5' terminal phosphate

FIGURE 28B

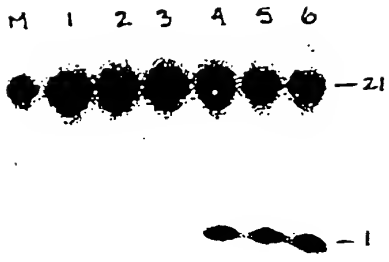


FIGURE 29

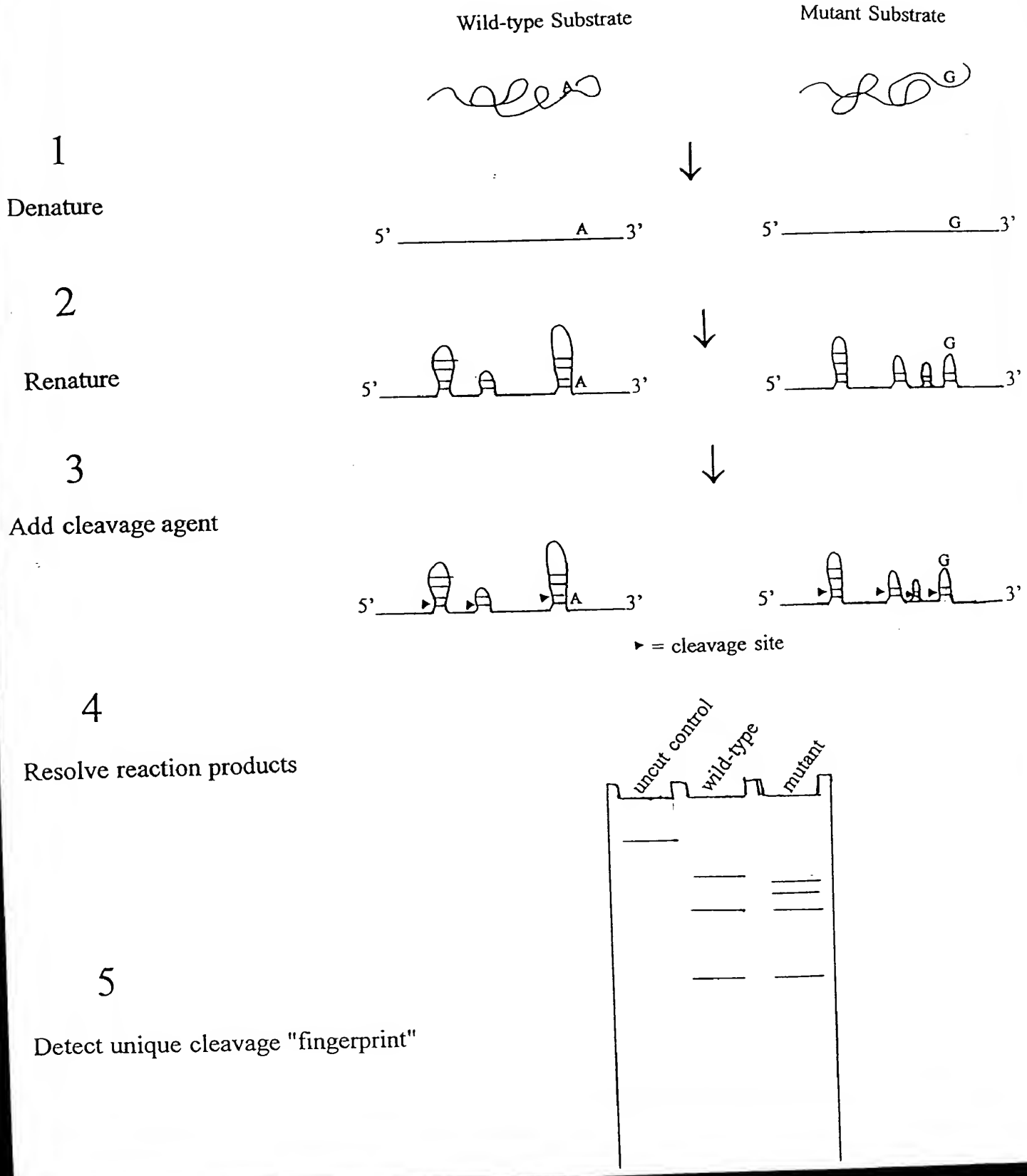

Wild-type Substrate
Mutant Substrate
A
G
1
Denature
5' A 3'
5' G 3'
2
Renature
5' A 3'
5' G 3'
3
Add cleavage agent
5' A 3'
5' G 3'
▸ = cleavage site
4
Resolve reaction products
uncut control
wild-type
mutant
5
Detect unique cleavage "fingerprint"

FIGURE 30

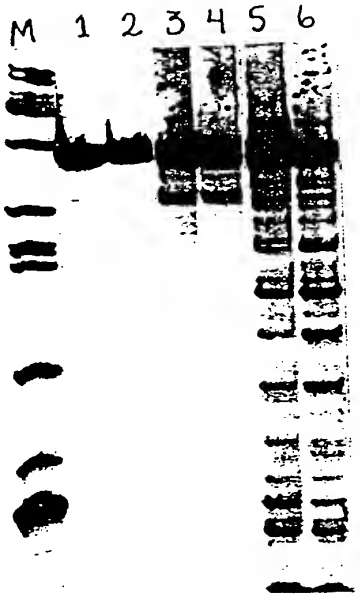


FIGURE 31

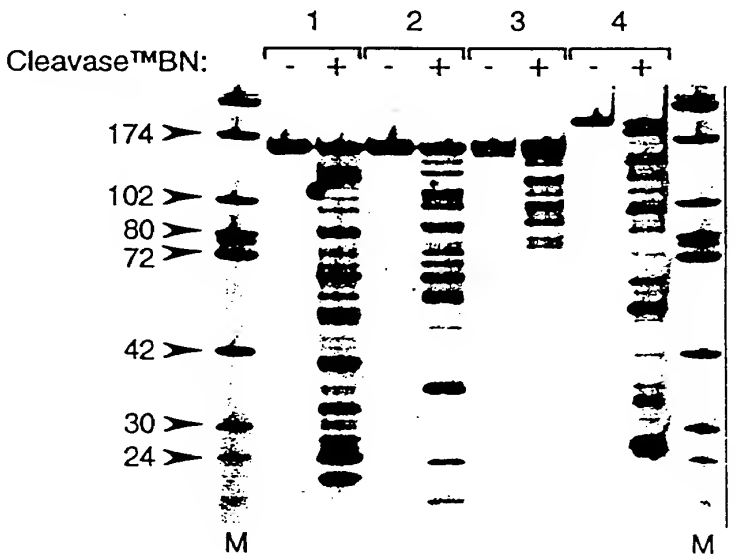


FIGURE 32

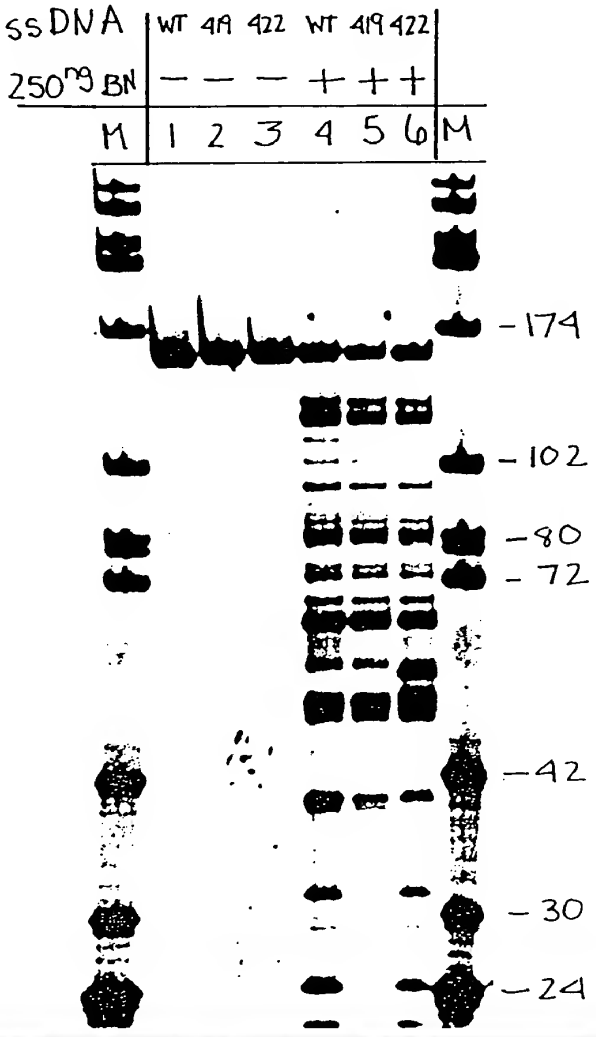

ssDNA
WT 419 422 WT 419 422
250ng BN
— — — + + +
M 1 2 3 4 5 6 M
174
102
80
72
42
30
24

FIGURE 33

FIGURE 34

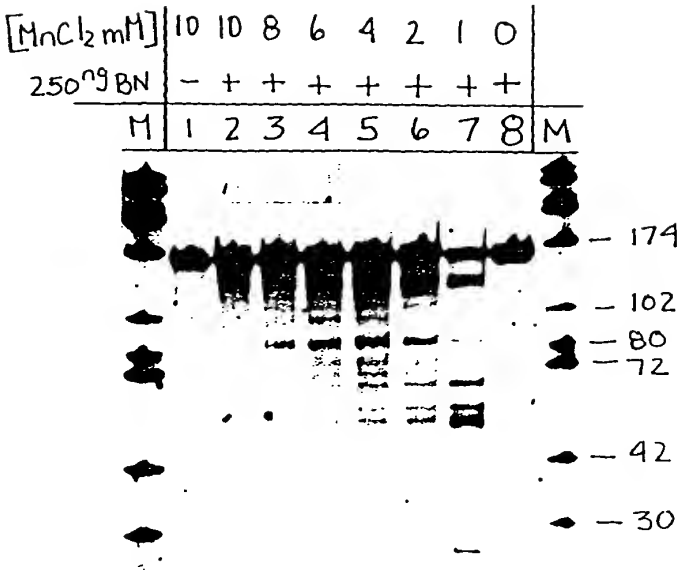


FIGURE 35

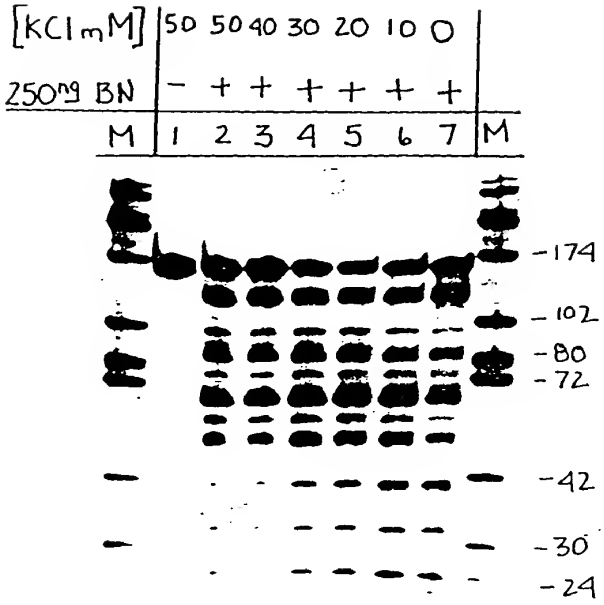

[KCl mM]
50 50 40 30 20 10 0
250ng BN
- + + + + + +
M
1 2 3 4 5 6 7
M
174
102
80
72
42
30
24

FIGURE 36

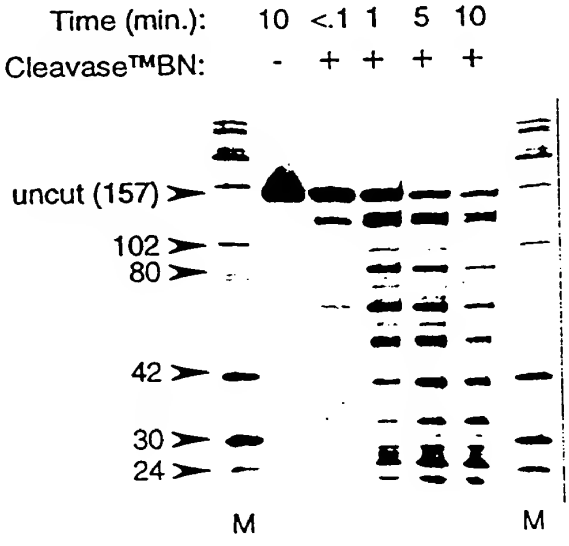


FIGURE 37

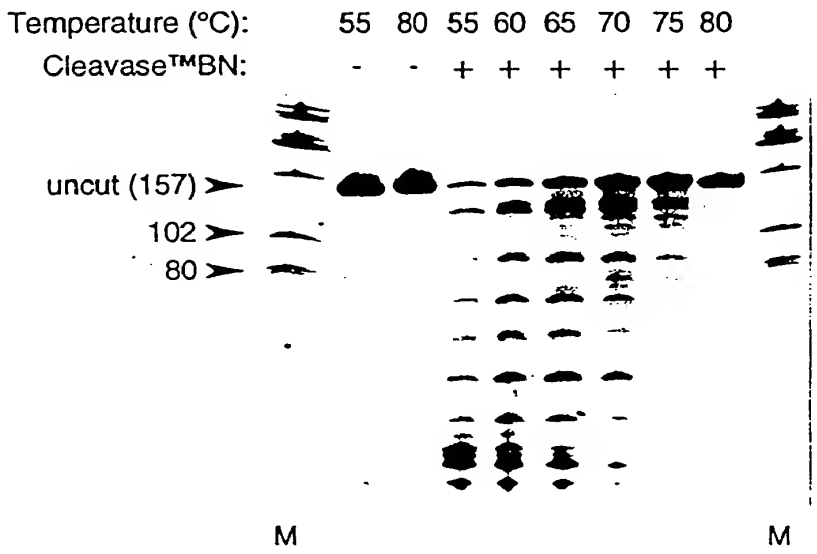

Temperature (°C): 55 80 55 60 65 70 75 80
Cleavase™BN: - - + + + + + +
uncut (157)
102
80
M
M

FIGURE 38

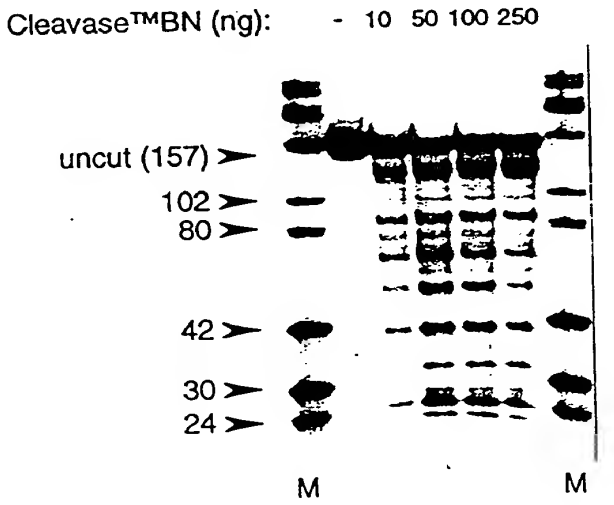


FIGURE 39

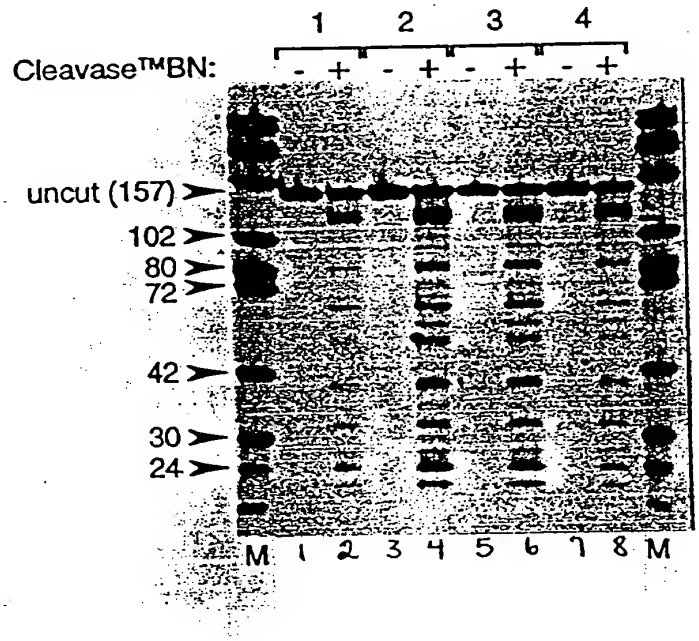


FIGURE 40

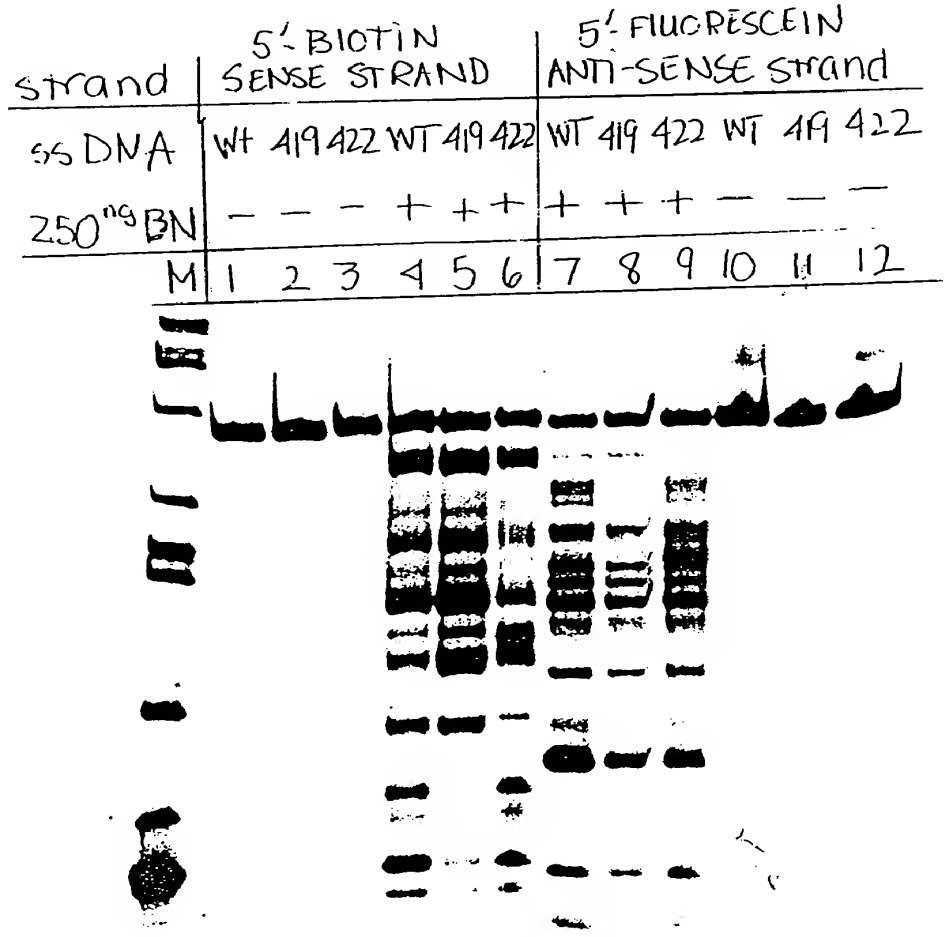

5'-BIOTIN
SENSE STRAND
5'-FLUORESCEIN
ANTI-SENSE STRAND
strand
ss DNA
250ng BN
WT 419 422 WT 419 422
WT 419 422 WT 419 422
M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 41

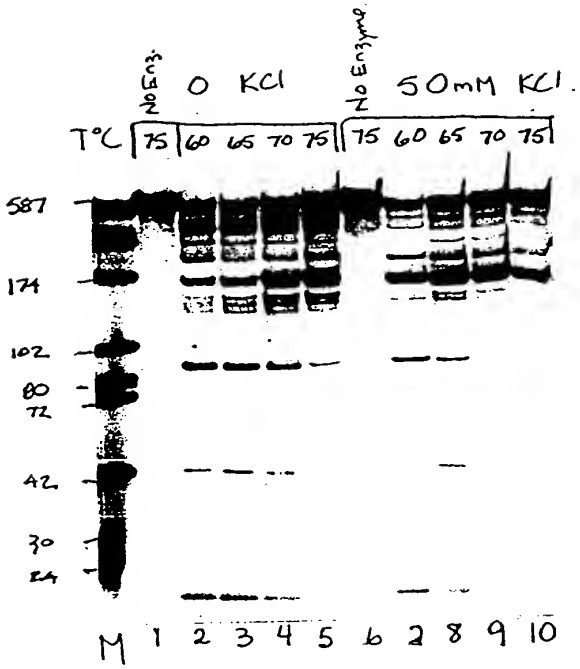

No Enzyme
0 KCl
No Enzyme
50mM KCl
T°C
75
60
65
70
75
75
60
65
70
75
587
174
102
80
72
42
30
M
1
2
3
4
5
6
7
8
9
10

FIGURE 42

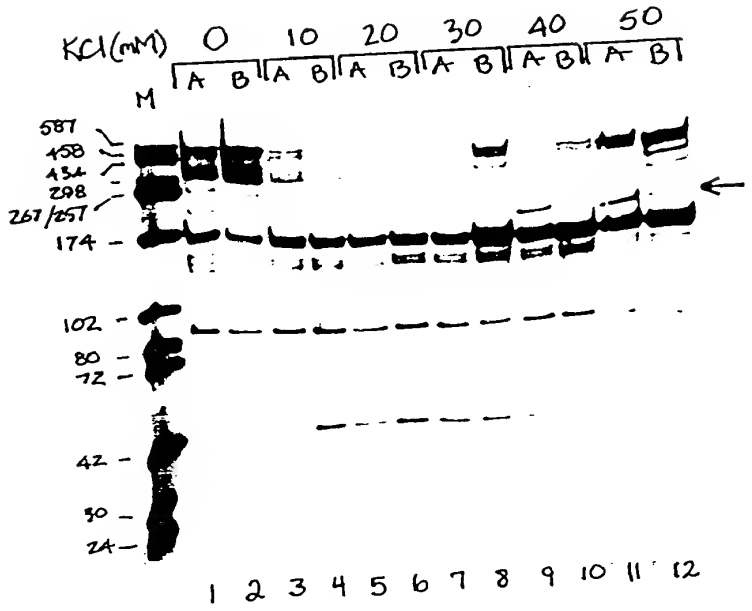

KCl(mM) 0 10 20 30 40 50
A B A B A B A B A B A B
M
587
488
434
298
267/257
174
102
80
72
42
30
24
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 43

FIGURE 44

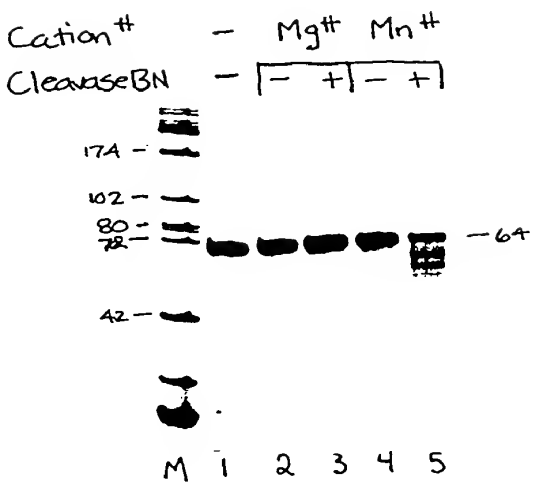


FIGURE 45

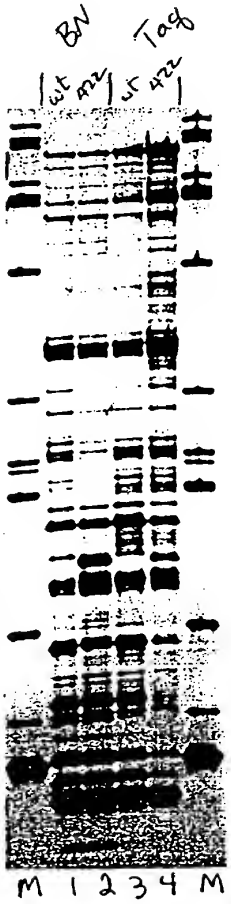


FIGURE 46

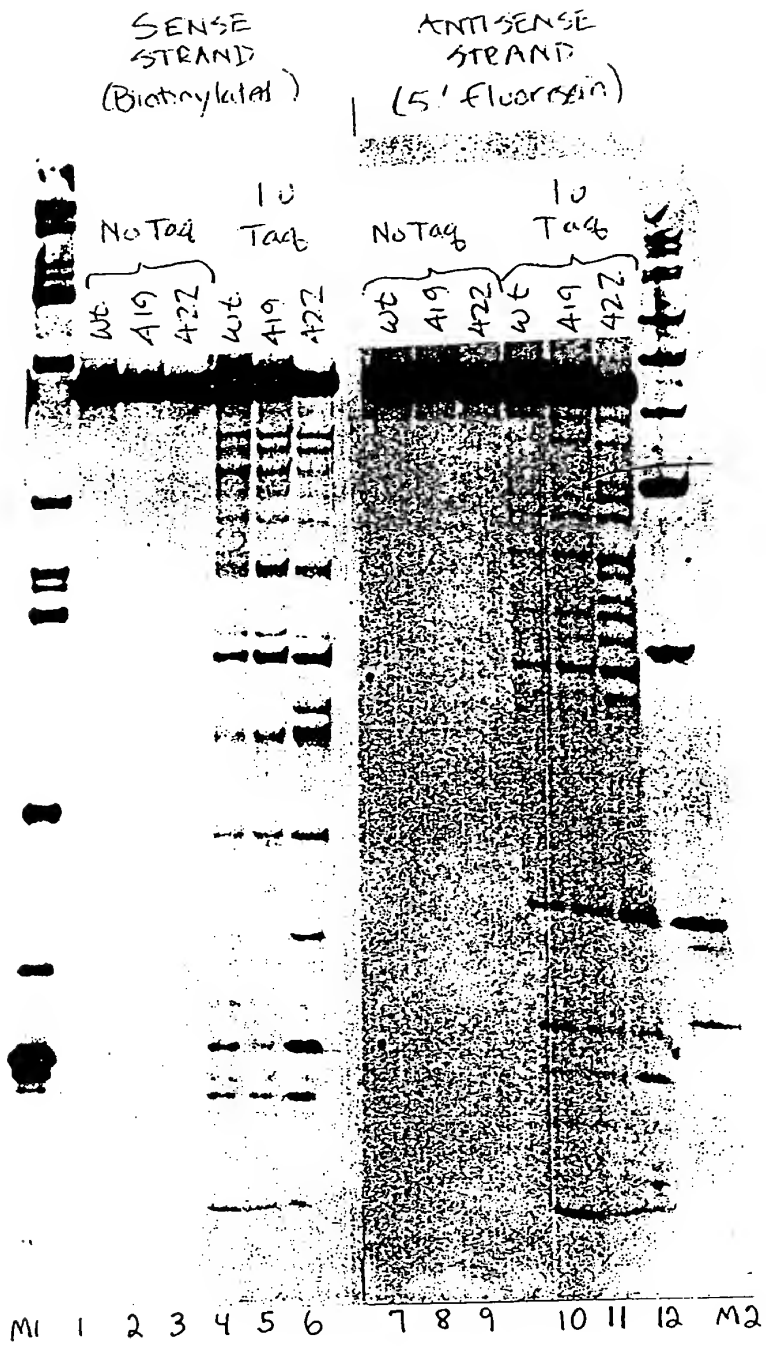

SENSE STRAND (Biotinylated)
ANTISENSE STRAND (5' Fluorescein)
No Taq
Taq
wt
419
422
M1 1 2 3 4 5 6 7 8 9 10 11 12 M2

FIGURE 47

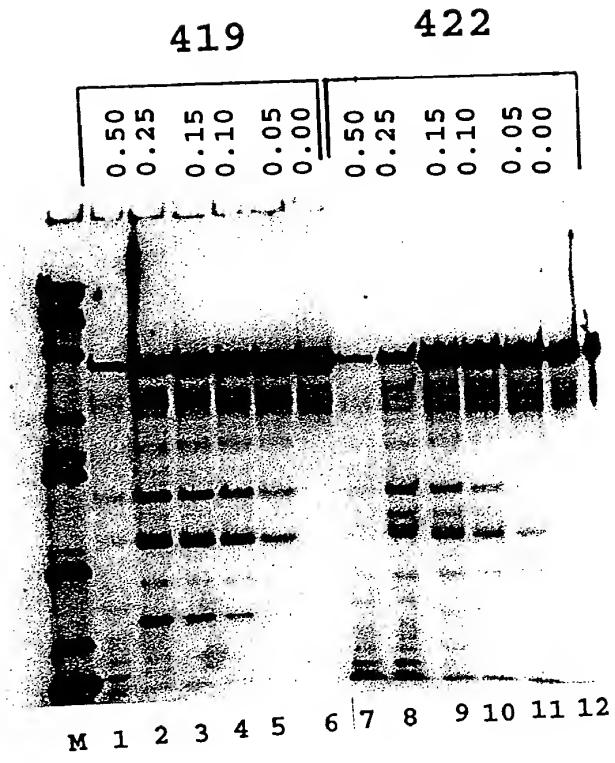

419
422
0.50
0.25
0.15
0.10
0.05
0.00
0.50
0.25
0.15
0.10
0.05
0.00
M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 48

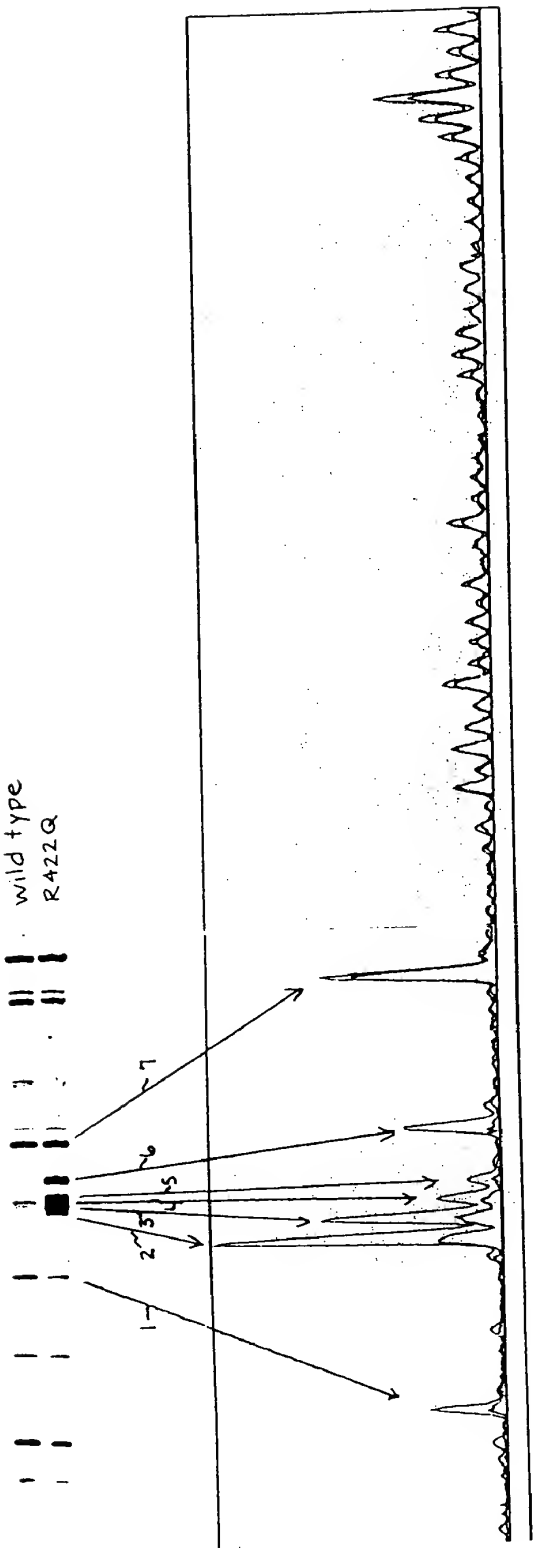


FIGURE 49

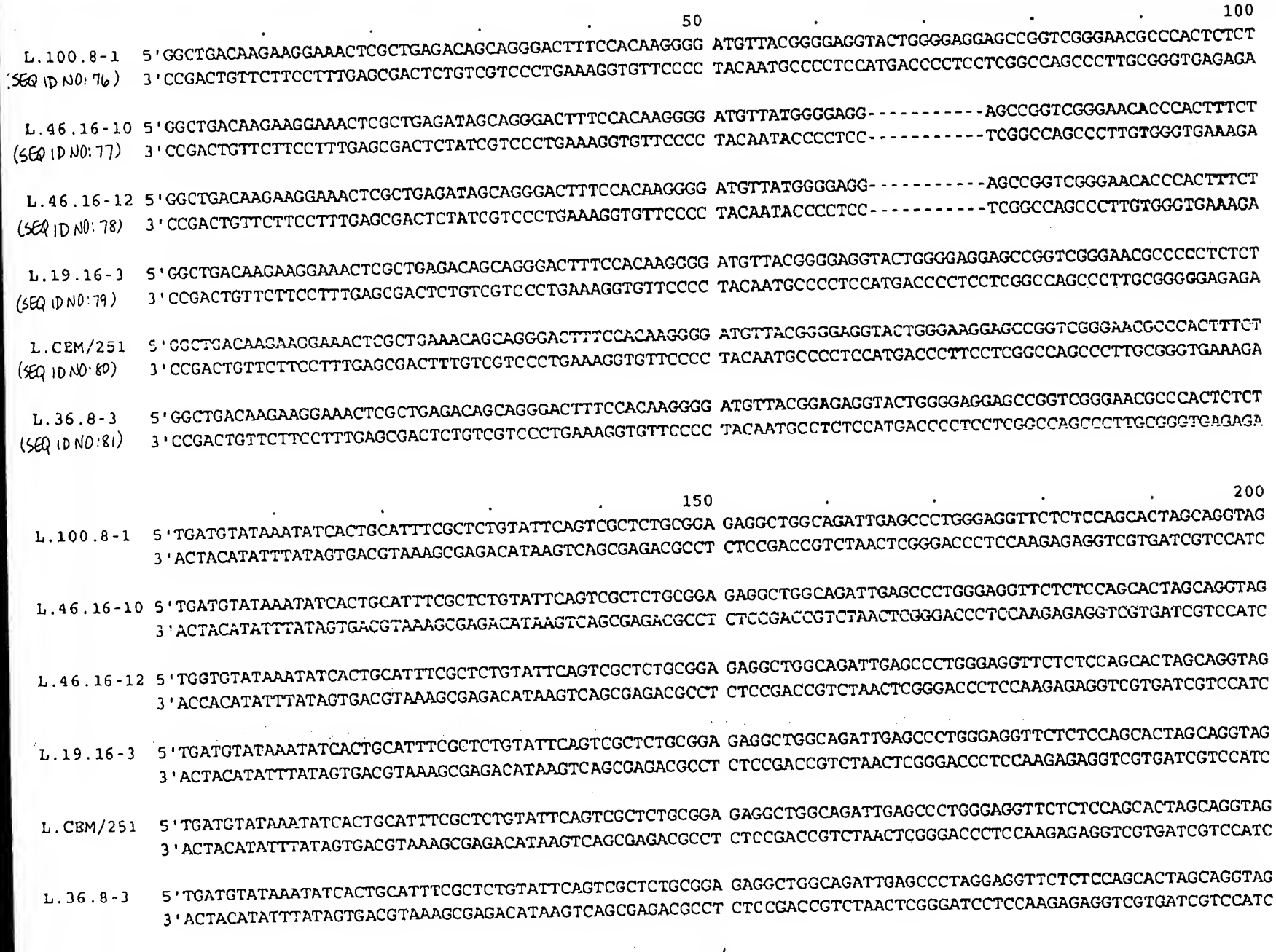


FIGURE 49

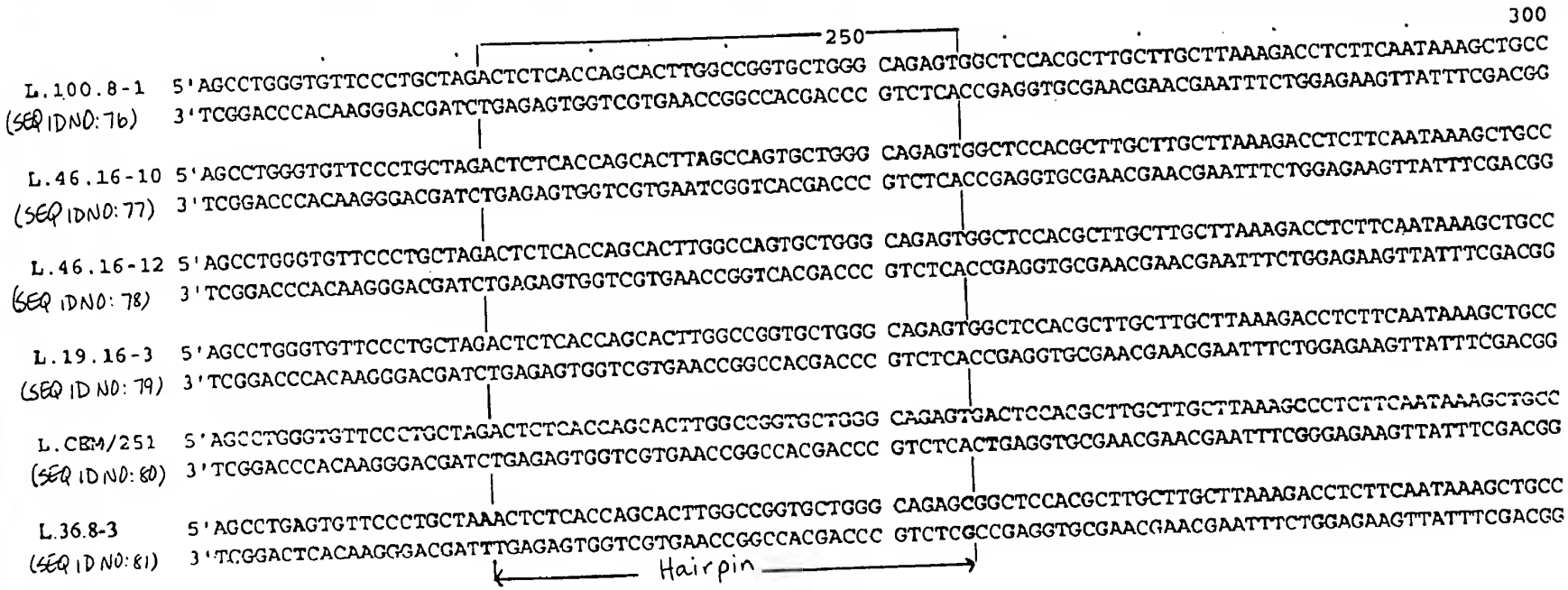


FIGURE 50

FIGURE 51

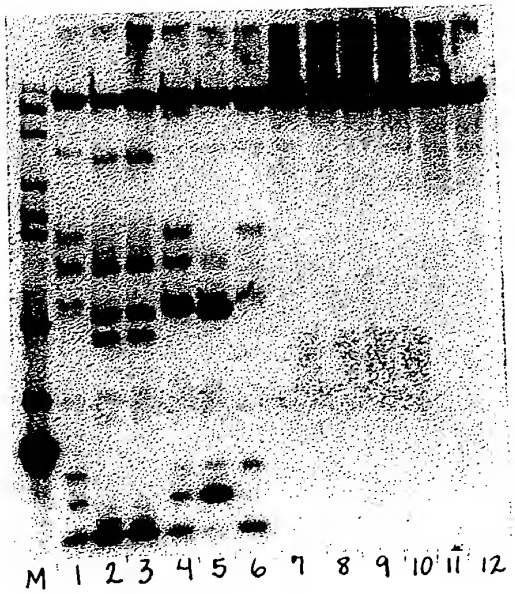


FIGURE 52

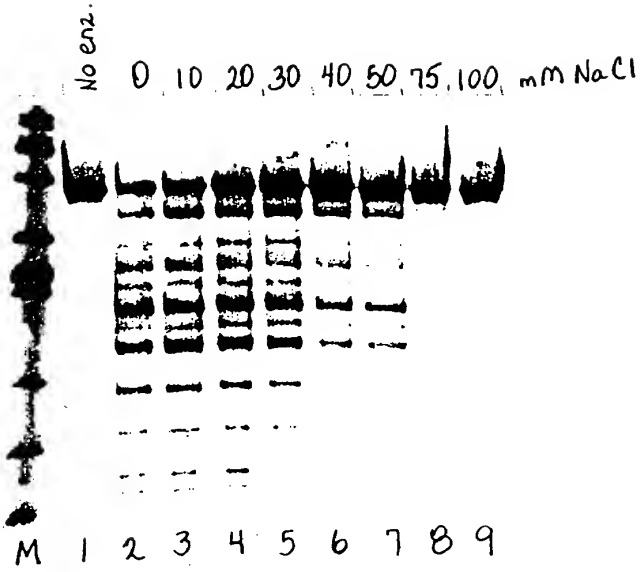


FIGURE 53

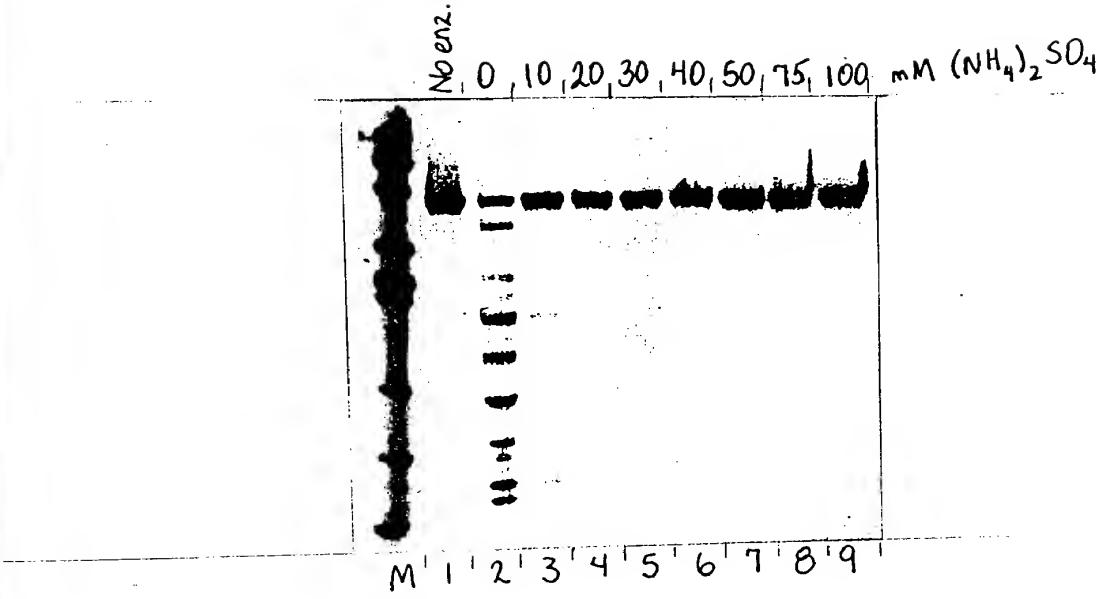


FIGURE 54

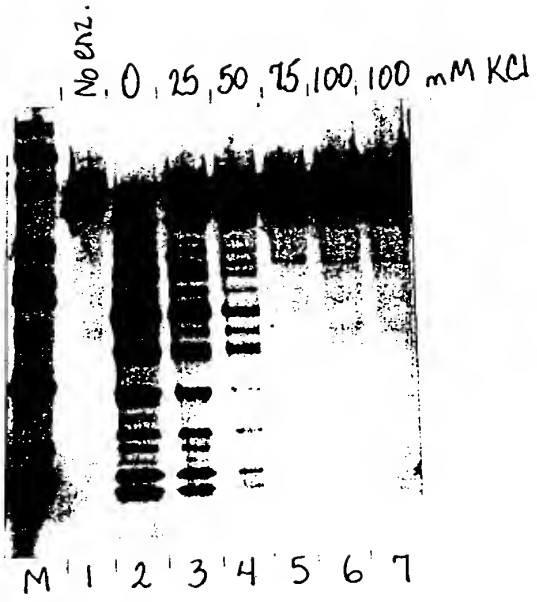


FIGURE 55

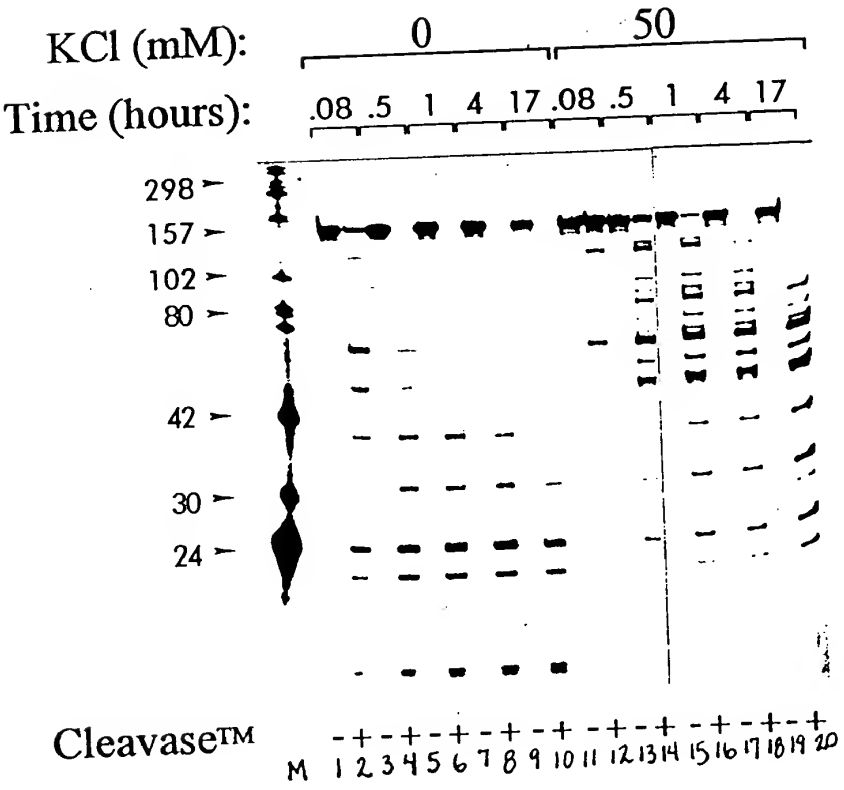

KCl (mM):
0
50
Time (hours):
.08 .5 1 4 17 .08 .5 1 4 17
298
157
102
80
42
30
24
Cleavase™
- + - + - + - + - + - + - + - + - + - + - +
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIGURE 56

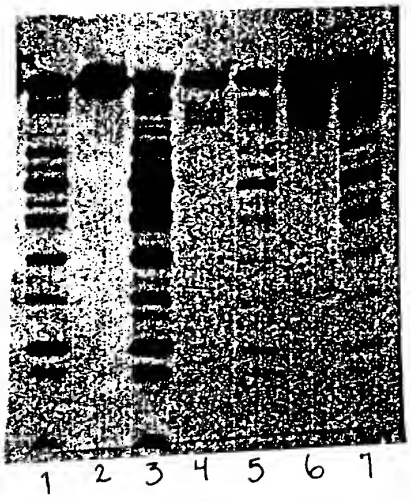


FIGURE 57

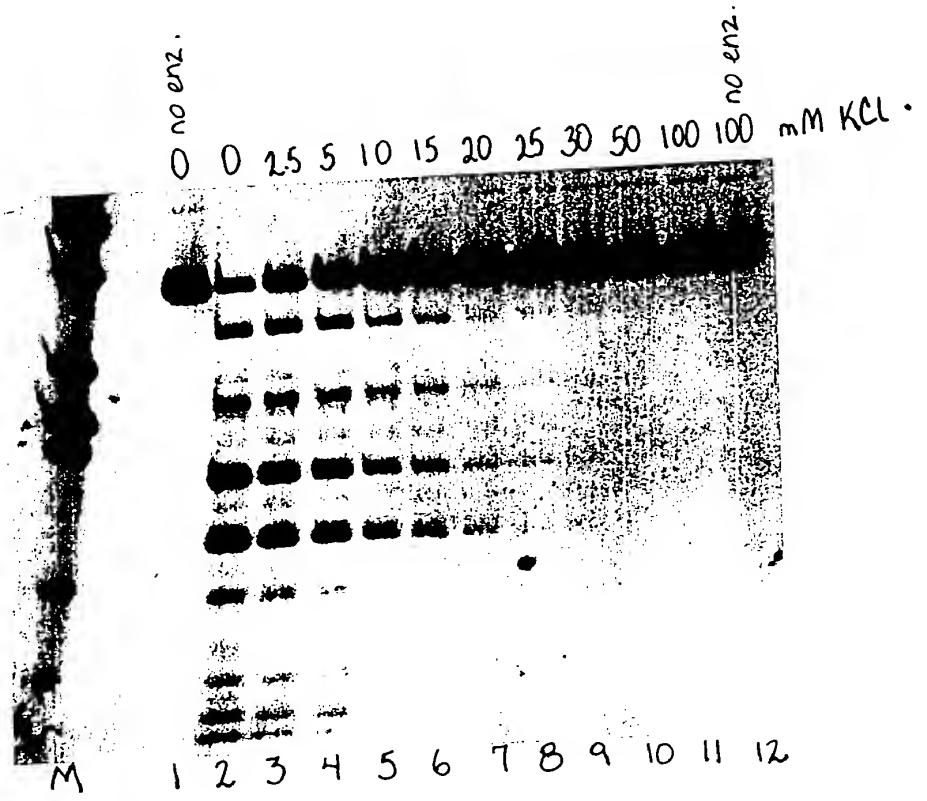

no enz.
0 0 2.5 5 10 15 20 25 30 50 100 100 mM KCl
no enz.
M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 58

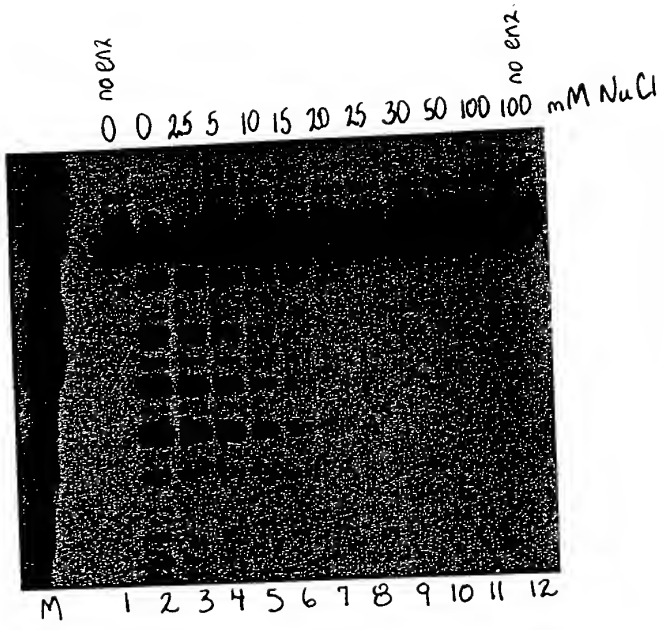

no enz
0 0 2.5 5 10 15 20 25 30 50 100 100 mM NaCl
no enz
M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 59

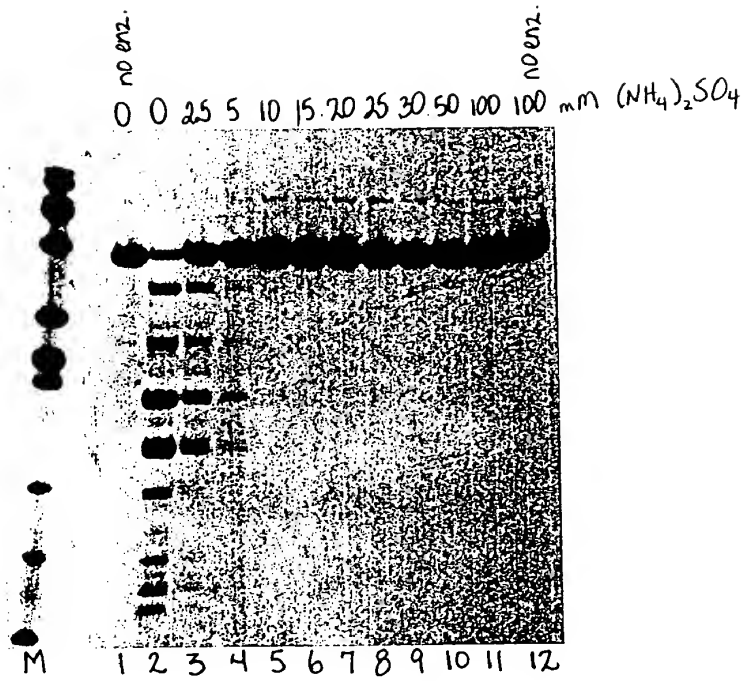

no enz.
no enz.
0 0 2.5 5 10 15 20 25 30 50 100 100 mM $(NH_4)_2SO_4$
M
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 60

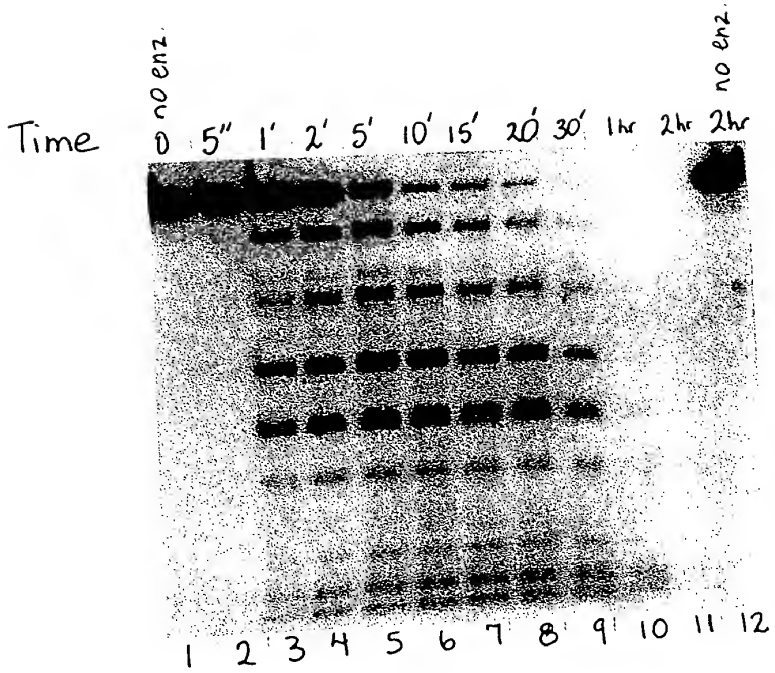

Time
no enz.
0
5"
1'
2'
5'
10'
15'
20'
30'
1hr
2hr
no enz.
2hr
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 61

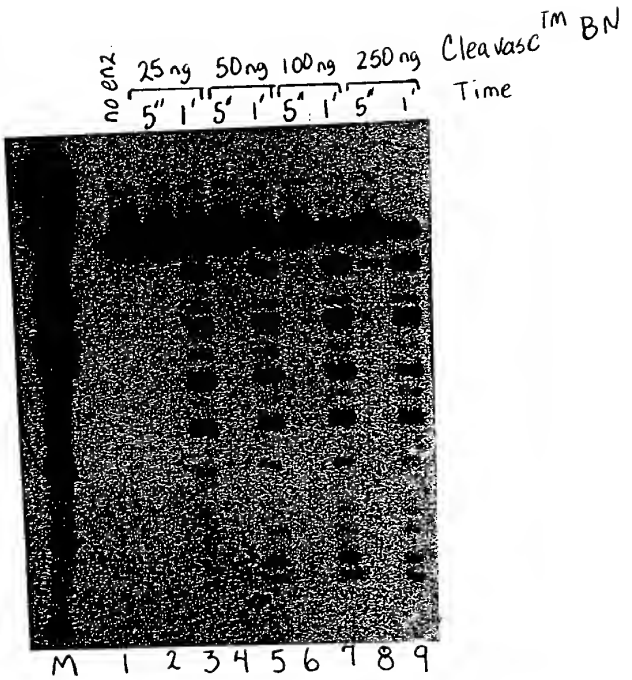

no enz
25 ng
50 ng
100 ng
250 ng
Cleavasc™ BN
5" 1' 5' 1' 5' 1' 5' 1'
Time
M 1 2 3 4 5 6 7 8 9

FIGURE 62

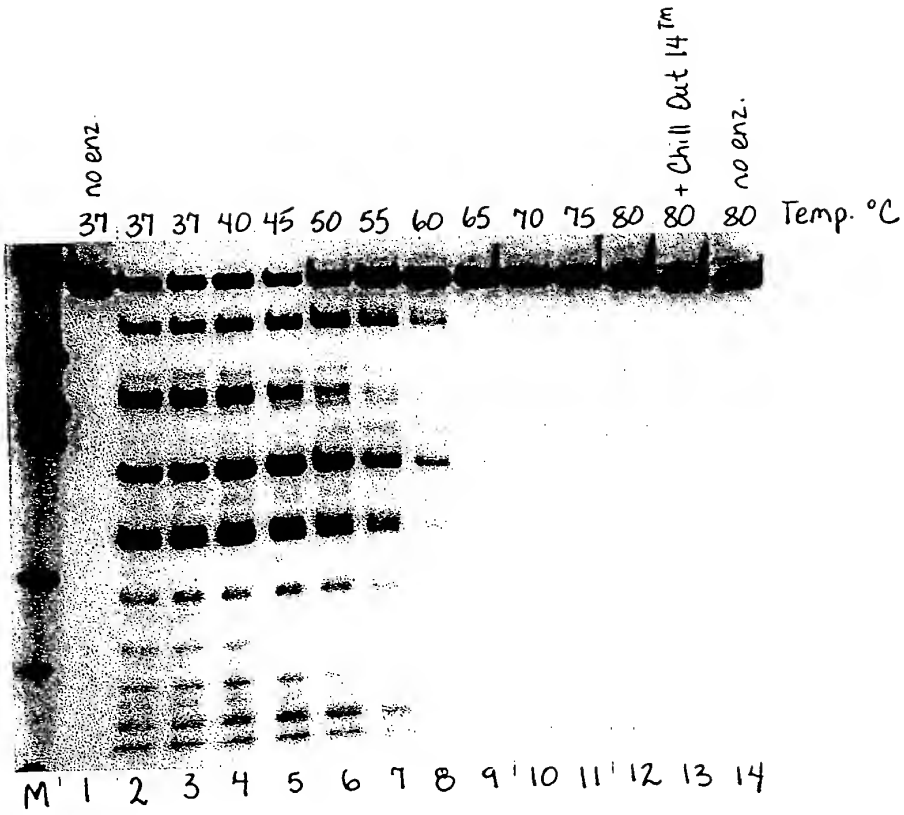

no enz
37 37 37 40 45 50 55 60 65 70 75 80 80 80
+ Chill Out 14™
no enz.
Temp. °C
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIGURE 63

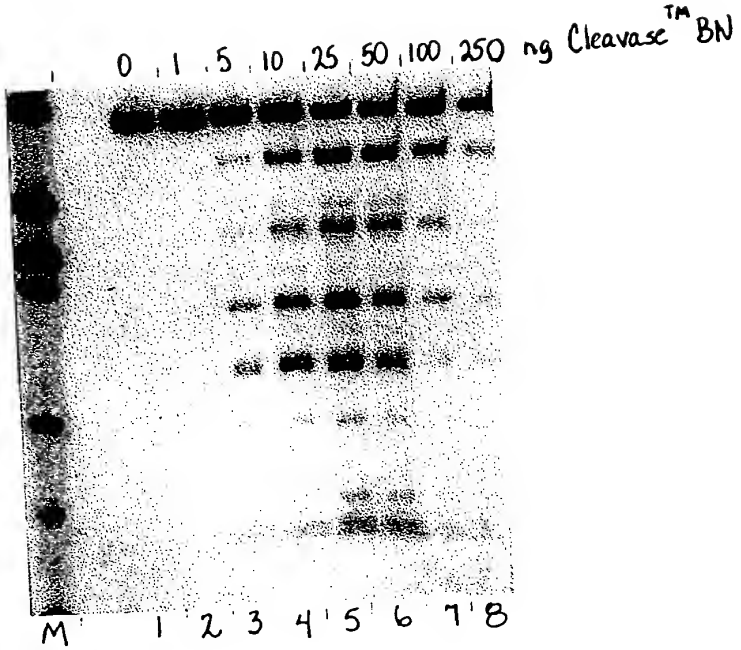
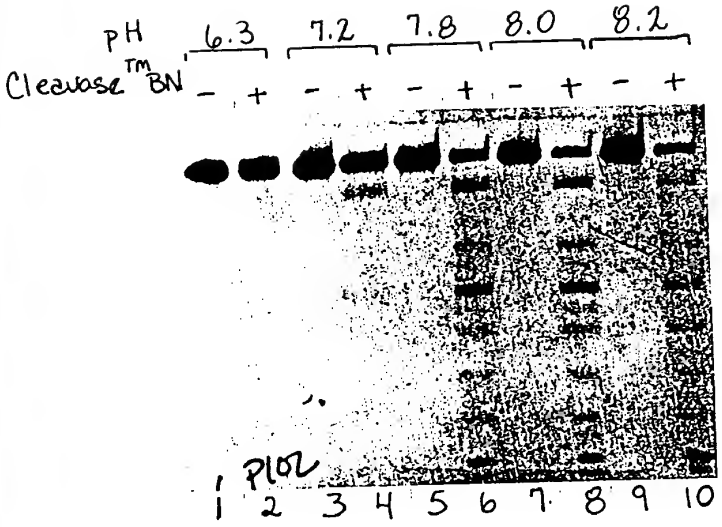


FIGURE 64

A


pH 6.3 7.2 7.8 8.0 8.2
Cleavase™ BN - + - + - + - + - +
P102
1 2 3 4 5 6 7 8 9 10

B

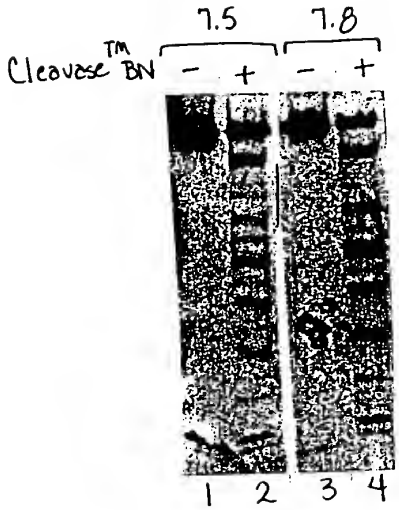
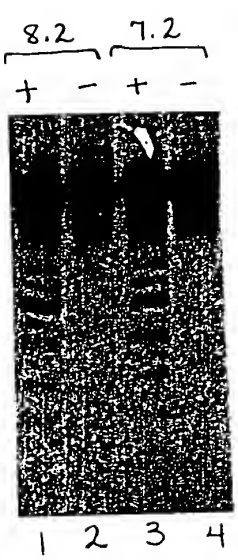

7.5 7.8
Cleavase™ BN - + - +
1 2 3 4

FIGURE 65

A



pH

Cleanse™ BN

B

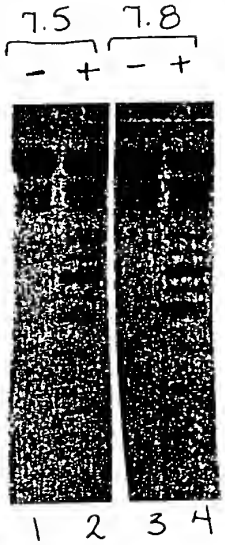


FIGURE 66

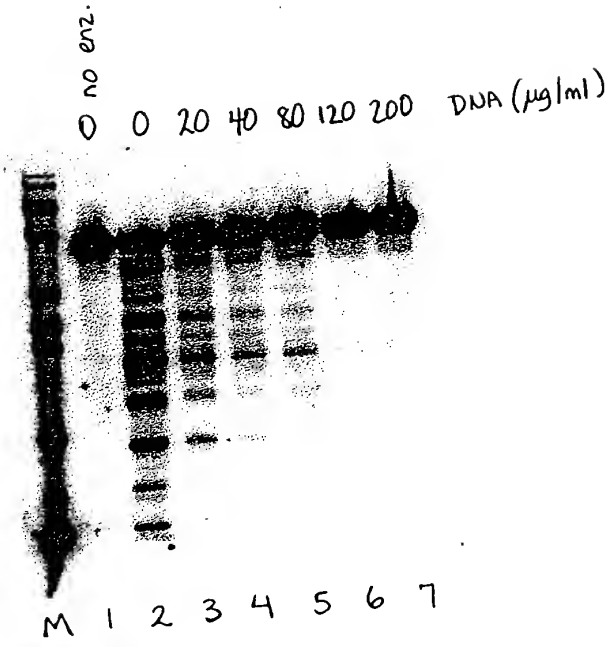


FIGURE 67

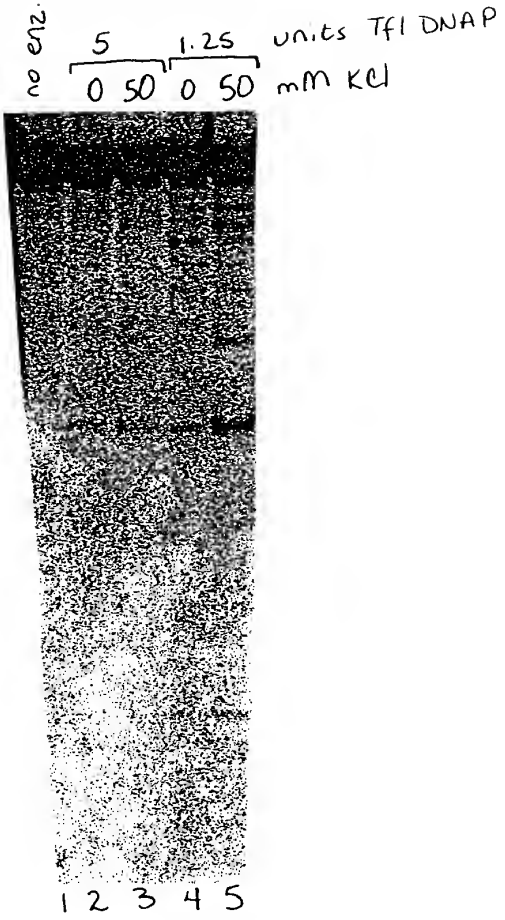


FIGURE 68

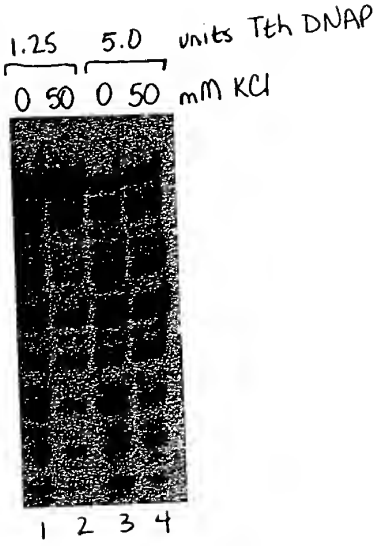


FIGURE 69

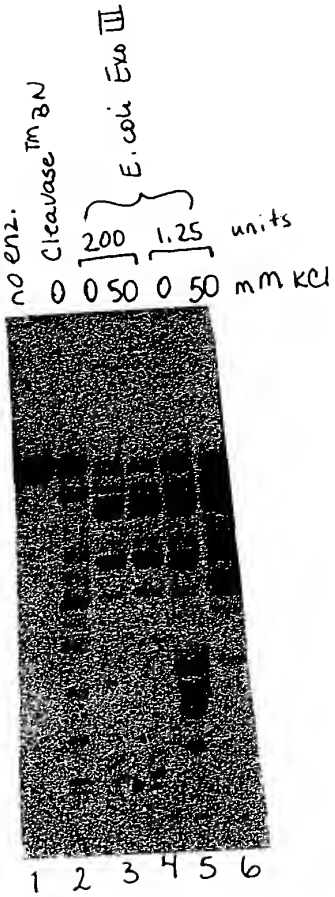

no enz.
Cleavase™ BN
E. coli Exo III
200
1.25
units
0 0 50 0 50 mM KCl
1 2 3 4 5 6

FIGURE 70

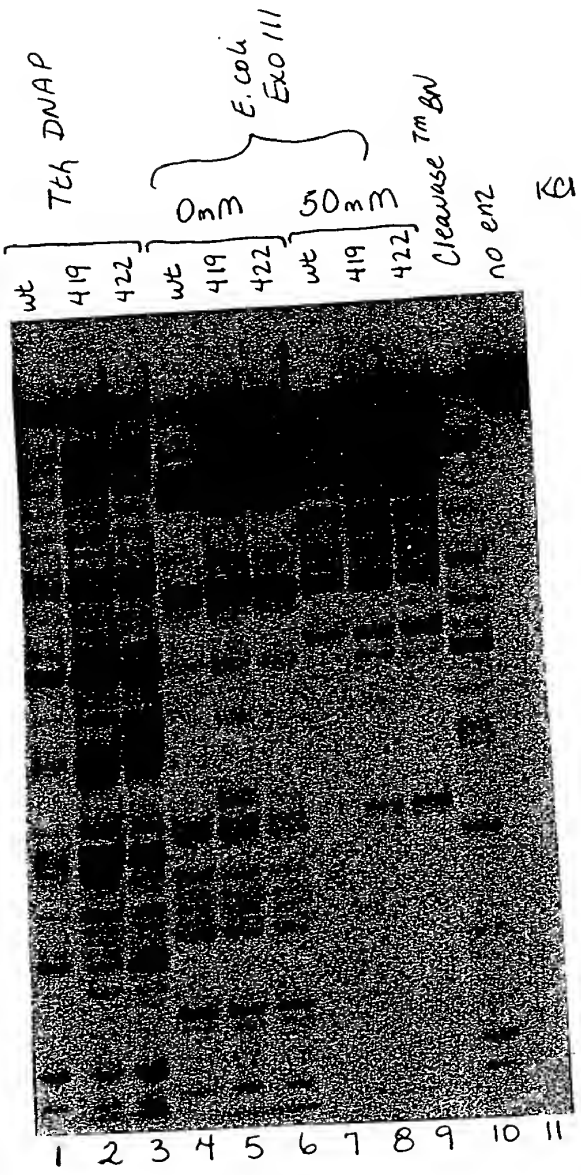

Tth DNAP
E. coli Exo III
0mM
50mM
wt
419
422
wt
419
422
wt
419
422
Cleavase™ BN
no enz
KCl
1 2 3 4 5 6 7 8 9 10 11

FIGURE 71

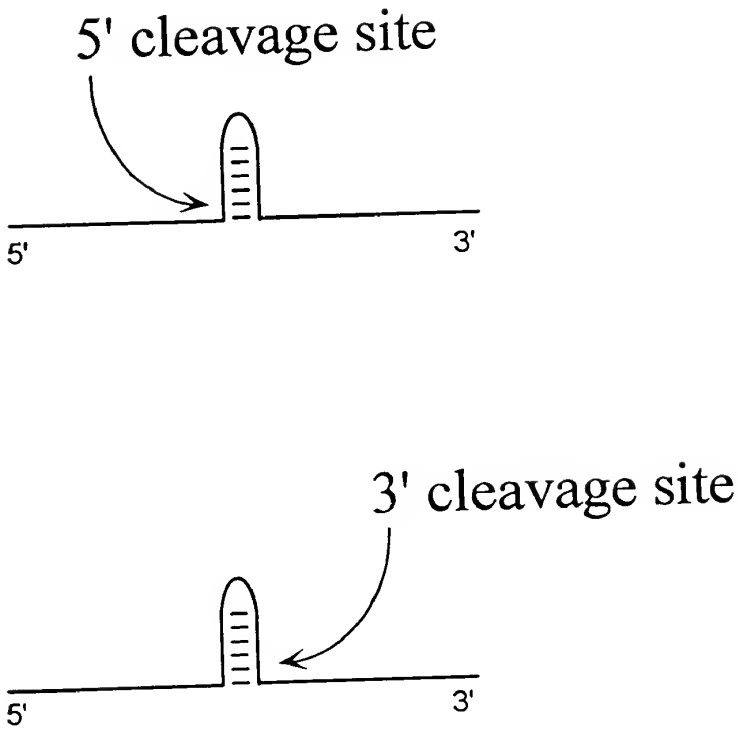


FIGURE 72

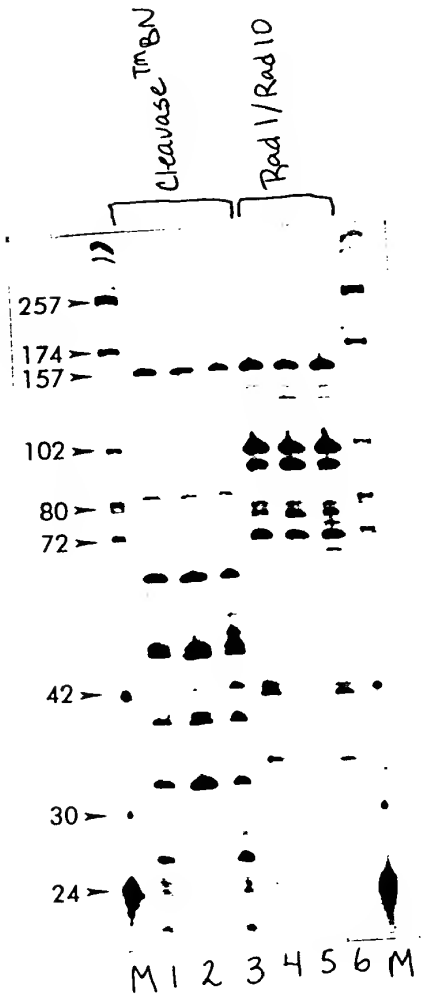
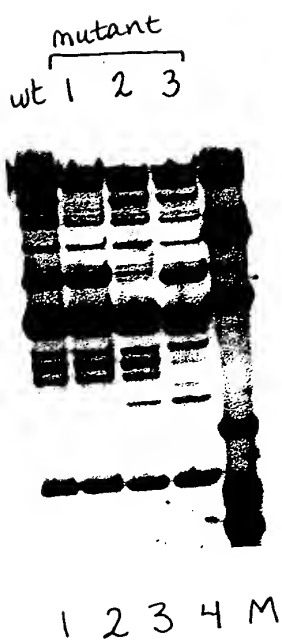


FIGURE 73

FIGURE 74

A



B

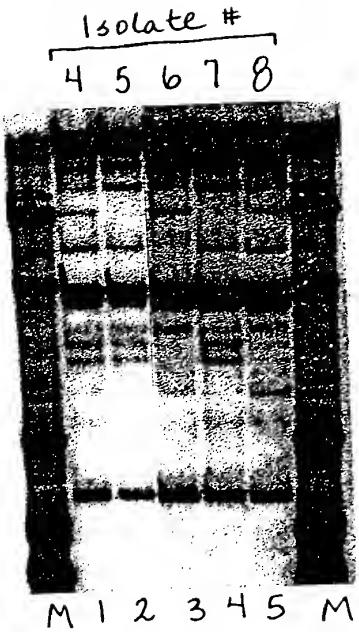


FIGURE 75

FIGURE 76

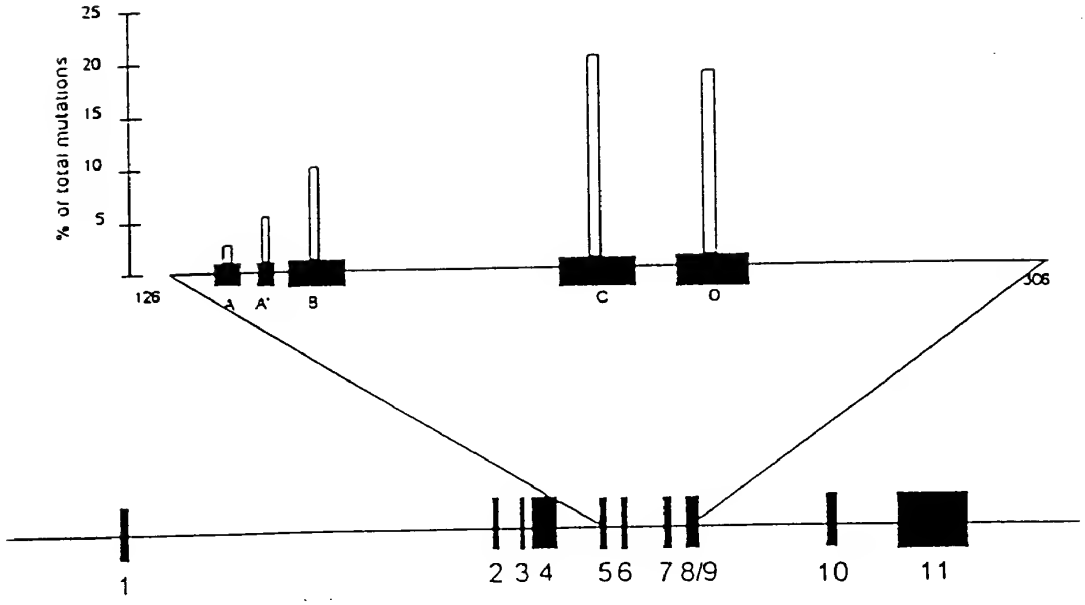


FIGURE 77

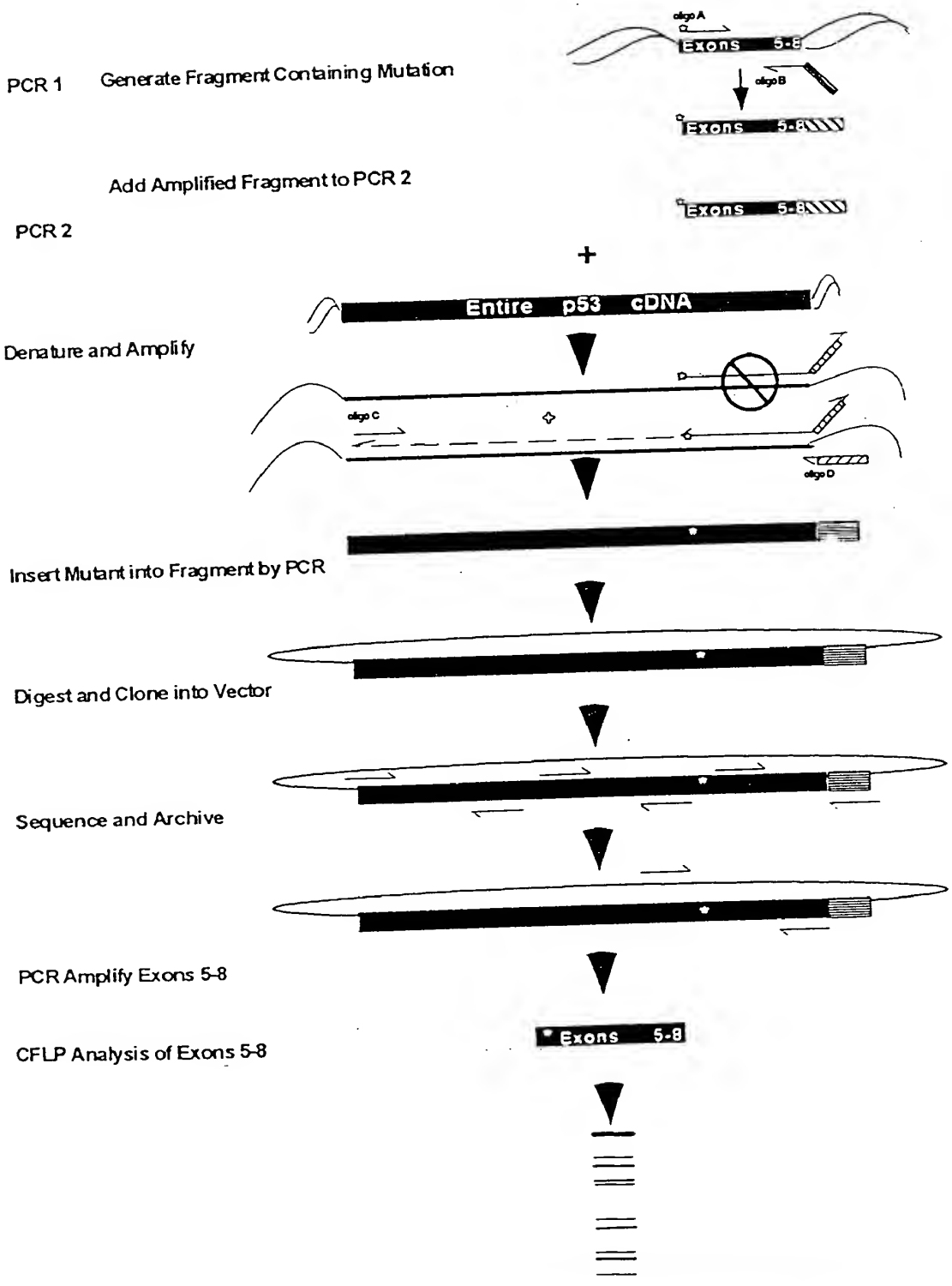

PCR 1
Generate Fragment Containing Mutation
oligo A
Exons 5-8
oligo B
Exons 5-8
Add Amplified Fragment to PCR 2
Exons 5-8
PCR 2
+
Entire p53 cDNA
Denature and Amplify
oligo C
oligo D
Insert Mutant into Fragment by PCR
Digest and Clone into Vector
Sequence and Archive
PCR Amplify Exons 5-8
Exons 5-8
CFLP Analysis of Exons 5-8

FIGURE 78

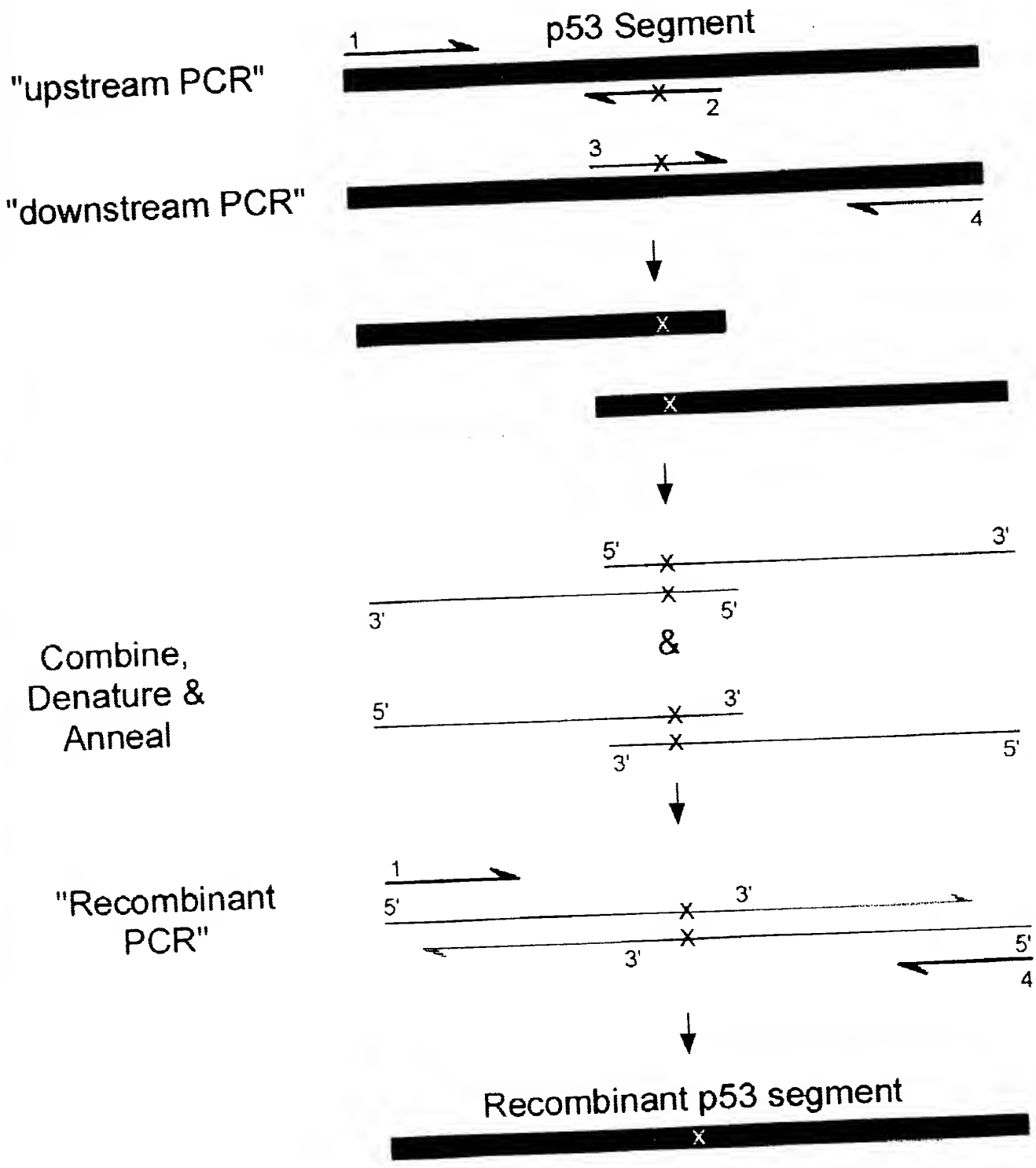

p53 Segment
"upstream PCR"
1
2
"downstream PCR"
3
4
X
X
5'
3'
3'
5'
&
Combine,
Denature &
Anneal
5'
3'
3'
5'
"Recombinant
PCR"
1
5'
3'
3'
5'
4
Recombinant p53 segment
X

FIGURE 79

FIGURE 80

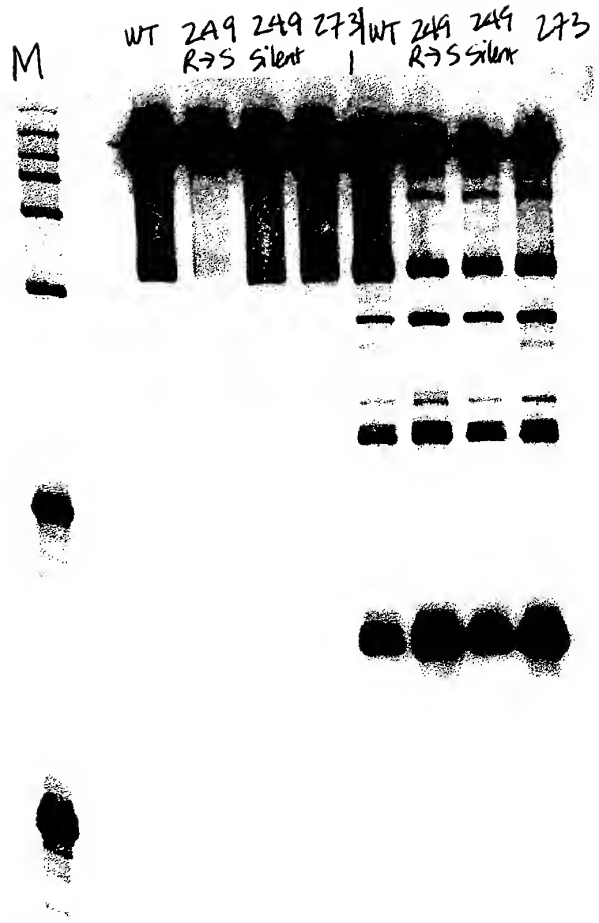


FIGURE 81

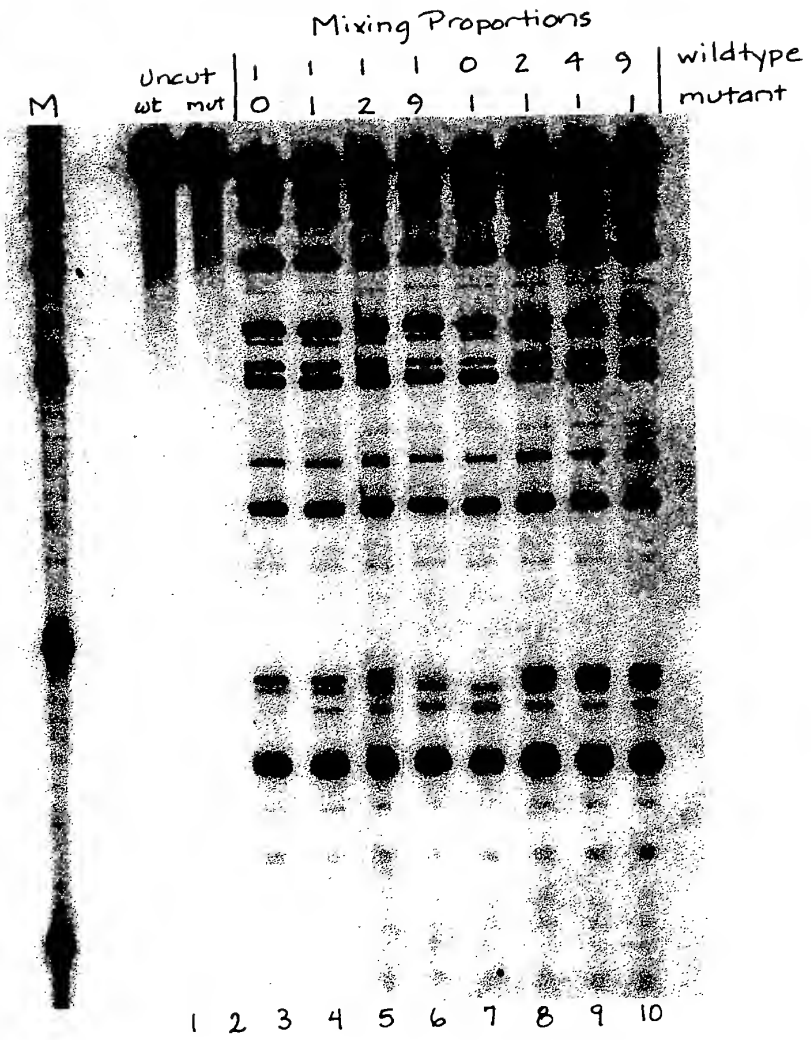

Mixing Proportions
M
Uncut
wt mut
1 1 1 1 0 2 4 9
0 1 2 9 1 1 1 1
wildtype
mutant
1 2 3 4 5 6 7 8 9 10

FIGURE 82

```
HCV1.1 (SEQ ID NO:121)
HCV2.1 (SEQ ID NO:122)
HCV3.1 (SEQ ID NO:123)
HCV4.2 (SEQ ID NO:124)
HCV6.1 (SEQ ID NO:125)
HCV7.1 (SEQ ID NO:126)

HCV1.1   1 CTGTCTTCAC GCAGAAAGCG TCTGGCCATG GCGTTAGTAT GAGTGTCGTG 50
HCV2.1     CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV3.1     CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV4.2     CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTA
HCV6.1     CTGTCTTCAC GCAGAAAGCG CCTAGCCATG GCGTTAGTAC GAGTGTCGTG
HCV7.1

HCV1.1  51 CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC 100
HCV2.1     CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV3.1     CAGCCTCCAG GTCCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV4.2     CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV6.1     CAGCCTCCAG GCCCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV7.1     CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC

HCV1.1  101 GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-AAA 150
HCV2.1      GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV3.1      GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV4.2      GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC GTGGATGTAA
HCV6.1      GGTGAGTACA CCGGAATTGC CGGGAAGACT GGGTCCTTTC TTGGAT-AAA
HCV7.1      GGTGAGTACA CCGGAATCGC TGGGGTGACC GGGTCCTTTC TTGGAG-CAA

HCV1.1  151 CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG 200
HCV2.1      CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV3.1      CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCGAGA CTGCTAGCCG
HCV4.2      CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV6.1      CCCACTCTAT GCCCGGCCAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV7.1      CCCGCTCAAT ACCCAGAAAT TTGGGCGTGC CCCCGCGAGA TCACTAGCCG

HCV1.1  201 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCCT 250
HCV2.1      AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV3.1      AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV4.2      AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV6.1      AGTAGCGTTG GGTTGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV7.1      AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT

HCV1.1  251 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC 282
HCV2.1      GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV3.1      GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV4.2      GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV6.1      GCGAGTACCC CGGGAGGTCT CGTAGACCGT GC
HCV7.1      GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
```

FIGURE 83

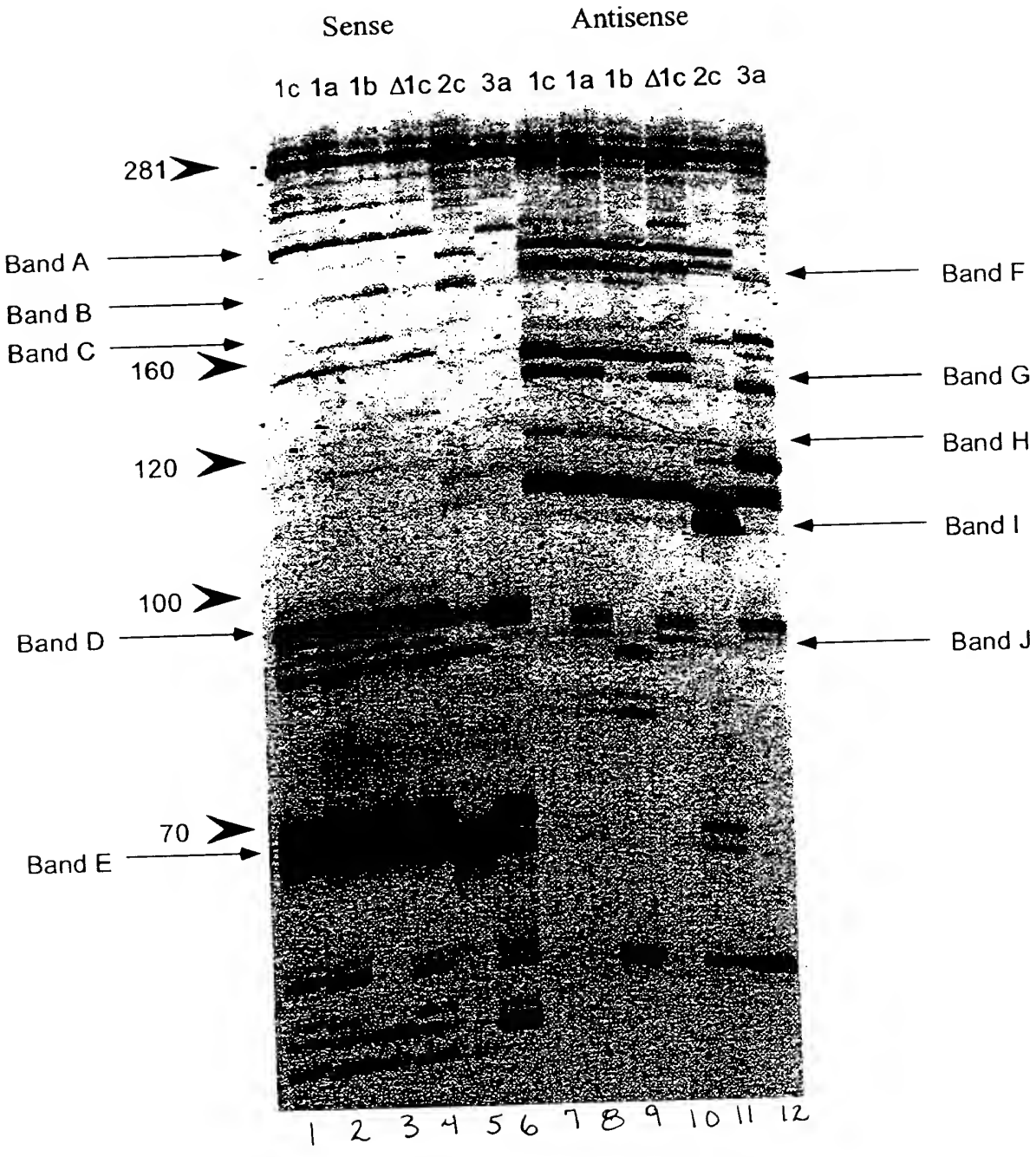

Sense
Antisense
1c 1a 1b Δ1c 2c 3a 1c 1a 1b Δ1c 2c 3a
281
Band A
Band B
Band C
160
120
100
Band D
70
Band E
Band F
Band G
Band H
Band I
Band J
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 84

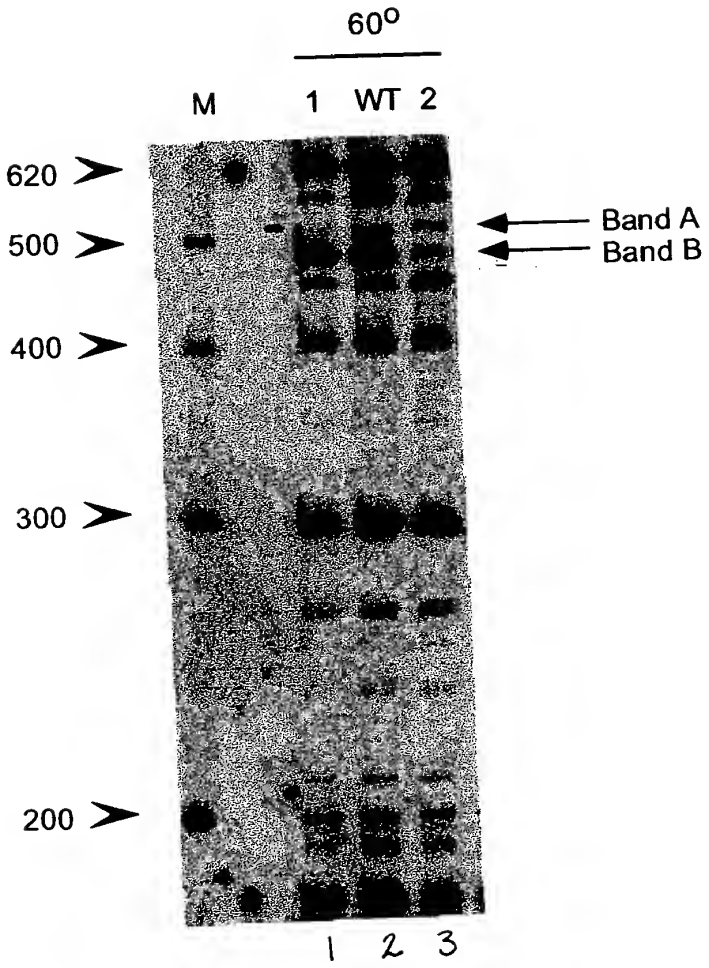


FIGURE 85

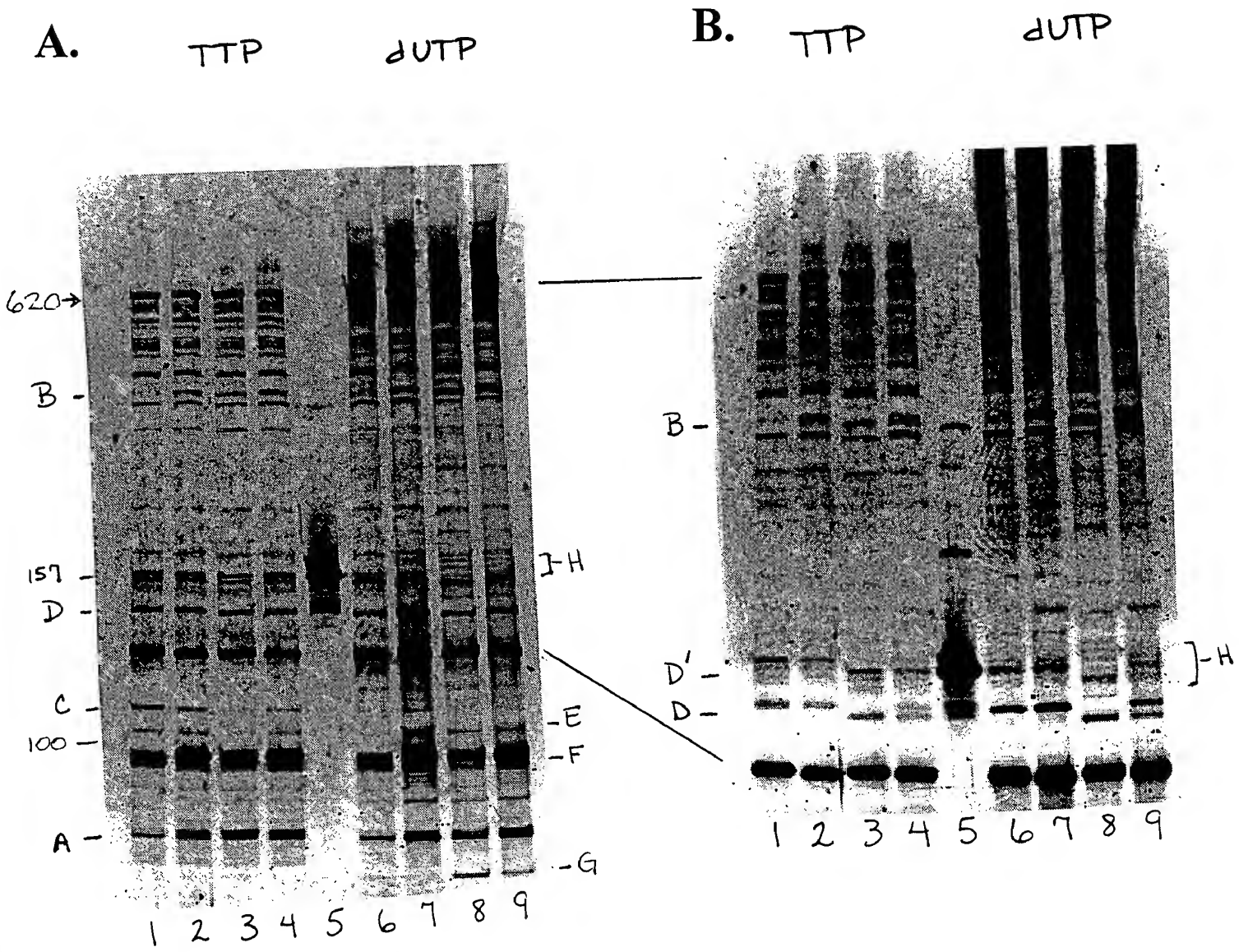


FIGURE 86

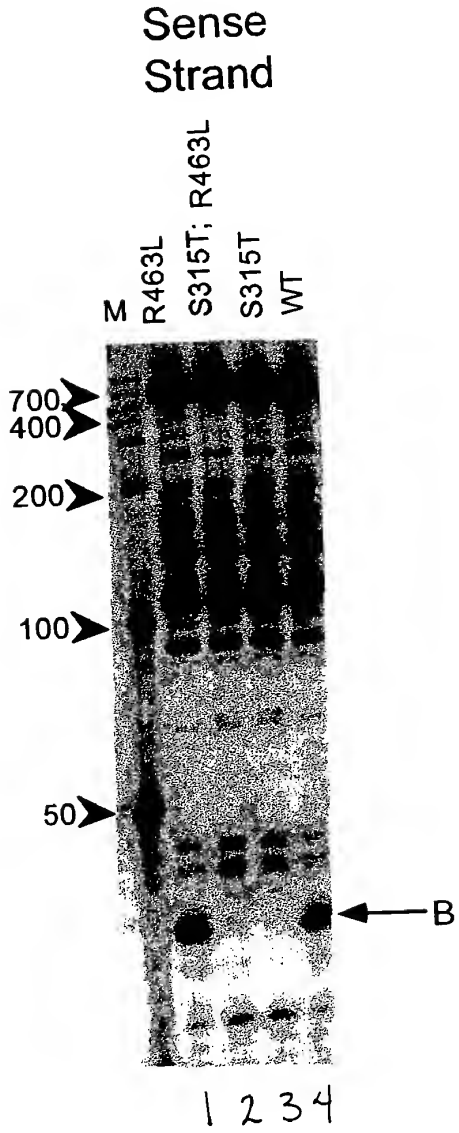

Sense
Strand
M
R463L
S315T; R463L
S315T
WT
700
400
200
100
50
B
1 2 3 4

FIGURE 87

Antisense
Strand

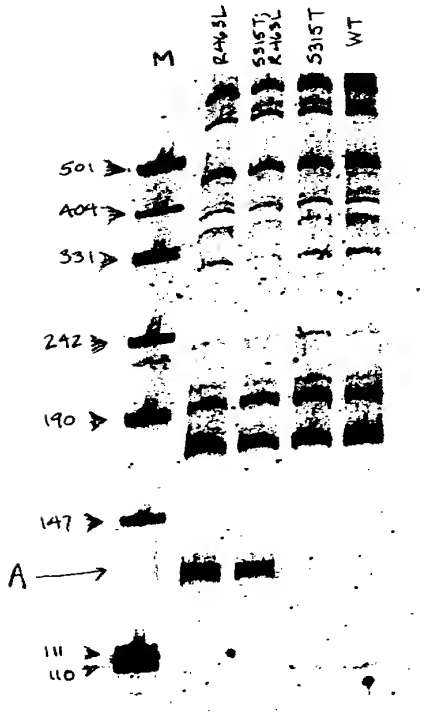


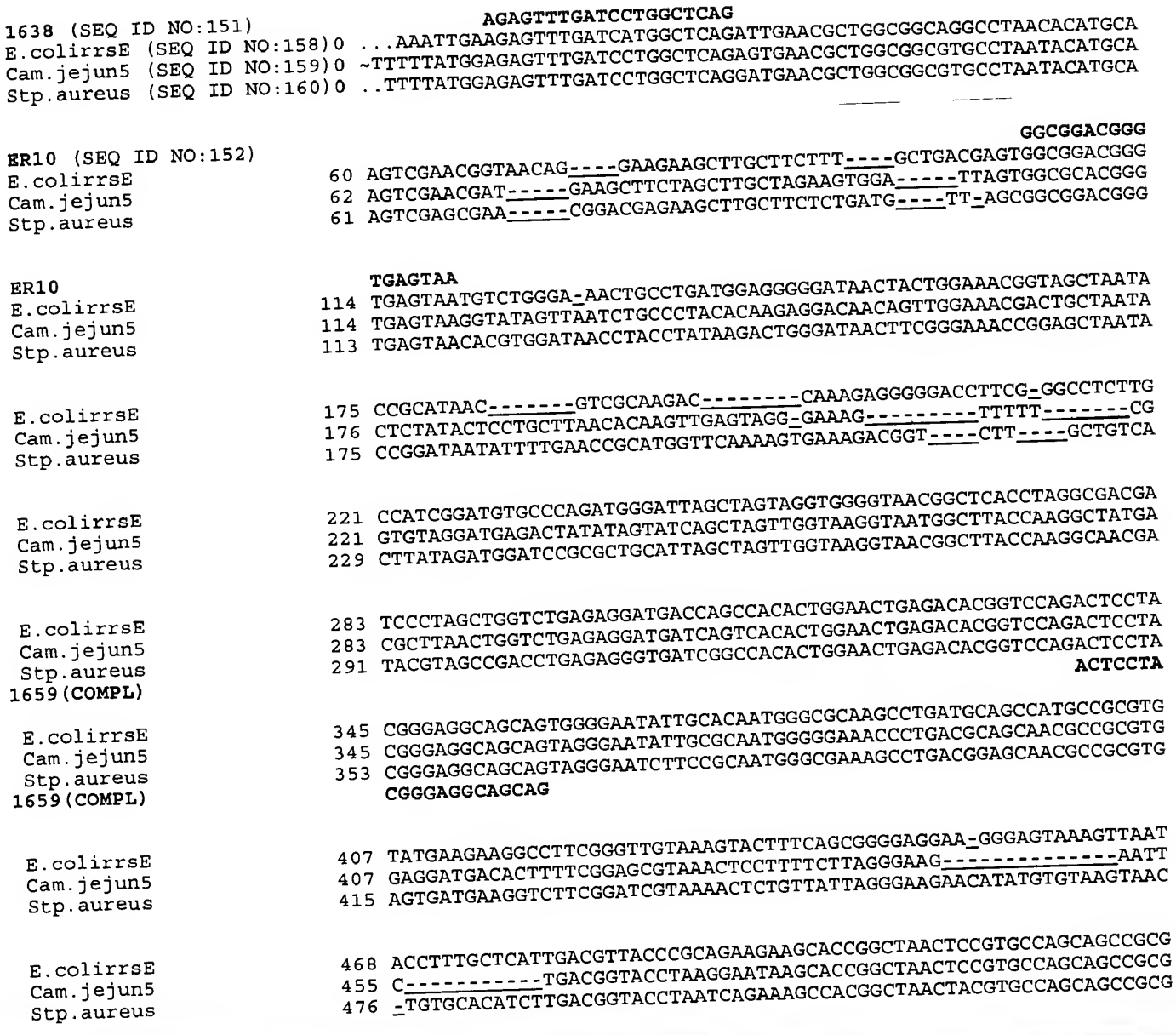
FIGURE 88


```
        10          20          30          40          50          60                    1638
       AGA GTTTGATCCT GGCTCAG
AAATTGAAGA GTTTGATCAT GGCTCAGATT GAACGCTGGC GGCAGGCCTA ACACATGCAA
TTTAACTTCT CAAACTAGTA CCGAGTCTAA CTTGCGACCG CCGTCCGGAT TGTGTACGTT

        70          80          90          100         110         120
                                                GGCGGAC GGGTGAGTAA                        ER10
GTCGAACGGT AACAGGAAGA AGCTTGCTTC TTTGCTGACG AGTGGCGGAC GGGTGAGTAA
CAGCTTGCCA TTGTCCTTCT TCGAACGAAG AAACGACTGC TCACCGCCTG CCCACTCATT

       130         140         150         160         170         180
TGTCTGGGAA ACTGCCTGAT GGAGGGGGAT AACTACTGGA AACGGTAGCT AATACCGCAT
ACAGACCCTT TGACGGACTA CCTCCCCCTA TTGATGACCT TTGCCATCGA TTATGGCGTA

       190         200         210         220         230         240
AACGTCGCAA GACCAAAGAG GGGGACCTTC GGGCCTCTTG CCATCGGATG TGCCCAGATG
TTGCAGCGTT CTGGTTTCTC CCCCTGGAAG CCCGGAGAAC GGTAGCCTAC ACGGGTCTAC

       250         260         270         280         290         300
GGATTAGCTA GTAGGTGGGG TAACGGCTCA CCTAGGCGAC GATCCCTAGC TGGTCTGAGA
CCTAATCGAT CATCCACCCC ATTGCCGAGT GGATCCGCTG CTAGGGATCG ACCAGACTCT

       310         320         330         340         350         360
GGATGACCAG CCACACTGGA ACTGAGACAC GGTCCAGACT CCTACGGGAG GCAGCAGTGG
CCTACTGGTC GGTGTGACCT TGACTCTGTG CCAGGTCTGA GGATGCCCTC CGTCGTCACC
                                  TGA GGATGCCCTC CGTCGTC                                  1659

       370         380         390         400         410         420
GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCCATGC CGCGTGTATG AAGAAGGCCT
CCTTATAACG TGTTACCCGC GTTCGGACTA CGTCGGTACG GCGCACATAC TTCTTCCGGA

       430         440         450         460         470         480
TCGGGTTGTA AAGTACTTTC AGCGGGGAGG AAGGGAGTAA AGTTAATACC TTTGCTCATT
AGCCCAACAT TTCATGAAAG TCGCCCCTCC TTCCCTCATT TCAATTATGG AAACGAGTAA

       490         500         510         520         530         540
GACGTTACCC GCAGAAGAAG CACCGGCTAA CTCCGTGCCA GCAGCCGCGG TAATACGGAG
CTGCAATGGG CGTCTTCTTC GTGGCCGATT GAGGCACGGT CGTCGGCGCC ATTATGCCTC

       550         560         570         580         590         600
GGTGCAAGCG TTAATCGGAA TTACTGGGCG TAAAGCGCAC GCAGGCGGTT TGTTAAGTCA
CCACGTTCGC AATTAGCCTT AATGACCCGC ATTTCGCGTG CGTCCGCCAA ACAATTCAGT

       610         620         630         640         650         660
GATGTGAAAT CCCCGGGCTC AACCTGGGAA CTGCATCTGA TACTGGCAAG CTTGAGTCTC
CTACACTTTA GGGGCCCGAG TTGGACCCTT GACGTAGACT ATGACCGTTC GAACTCAGAG

       670         680         690         700         710         720
GTAGAGGGGG GTAGAATTCC AGGTGTAGCG GTGAAATGCG TAGAGATCTG GAGGAATACC
CATCTCCCCC CATCTTAAGG TCCACATCGC CACTTTACGC ATCTCTAGAC CTCCTTATGG

       730         740         750         760         770         780
GGTGGCGAAG GCGGCCCCCT GGACGAAGAC TGACGCTCAG GTGCGAAAGC GTGGGGAGCA
CCACCGCTTC CGCCGGGGGA CCTGCTTCTG ACTGCGAGTC CACGCTTTCG CACCCCTCGT
```

FIGURE 88 Sheet 2/2

```
      790        800        810        820        830        840
AACAGGATTA GATACCCTGG TAGTCCACGC CGTAAACGAT GTCGACTTGG AGGTTGTGCC
TTGTCCTAAT CTATGGGACC ATCAGGTGCG GCATTTGCTA CAGCTGAACC TCCAACACGG

      850        860        870        880        890        900
CTTGAGGCGT GGCTTCCGGA GCTAACGCGT TAAGTCGACC GCCTGGGGAG TACGGCCGCA
GAACTCCGCA CCGAAGGCCT CGATTGCGCA ATTCAGCTGG CGGACCCCTC ATGCCGGCGT

      910        920        930        940        950        960
AGGTTAAAAC TCAAATGAAT TGACGGGGGC CCGCACAAGC GGTGGAGCAT GTGGTTTAAT
TCCAATTTTG AGTTTACTTA ACTGCCCCCG GGCGTGTTCG CCACCTCGTA CACCAAATTA

      970        980        990       1000       1010       1020
TCGATGCAAC GCGAAGAACC TTACCTGGTC TTGACATCCA CGGAAGTTTT CAGAGATGAG
AGCTACGTTG CGCTTCTTGG AATGGACCAG AACTGTAGGT GCCTTCAAAA GTCTCTACTC

      1030       1040       1050       1060       1070       1080
AATGTGCCTT CGGGAACCGT GAGACAGGTG CTGCATGGCT GTCGTCAGCT CGTGTTGTGA
TTACACGGAA GCCCTTGGCA CTCTGTCCAC GACGTACCGA CAGCAGTCGA GCACAACACT

      1090       1100       1110       1120       1130       1140
                 GC AACGAGCGCA ACCC                                    SB-1
AATGTTGGGT TAAGTCCCGC AACGAGCGCA ACCCTTATCC TTTGTTGCCA GCGGTCCGGC
TTACAACCCA ATTCAGGGCG TTGCTCGCGT TGGGAATAGG AAACAACGGT CGCCAGGCCG

      1150       1160       1170       1180       1190       1200
                                                  ATG ACGTCAAGTC      SB-3
                                                  ATG ACGTCAAGTC      SB-4
CGGGAACTCA AAGGAGACTG CCAGTGATAA ACTGGAGGAA GGTGGGGATG ACGTCAAGTC
GCCCTTGAGT TTCCTCTGAC GGTCACTATT TGACCTCCTT CCACCCCTAC TGCAGTTCAG

      1210       1220       1230       1240       1250       1260
                                                                      SB-3
                                                                      SB-4
ATCATGGCCC TTA
ATCATGGCCC TTACGA
ATCATGGCCC TTACGACCAG GGCTACACAC GTGCTACAAT GGCGCATACA AAGAGAAGCG
TAGTACCGGG AATGCTGGTC CCGATGTGTG CACGATGTTA CCGCGTATGT TTCTCTTCGC

      1270       1280       1290       1300       1310       1320
ACCTCGCGAG AGCAAGCGGA CCTCATAAAG TGCGTCGTAG TCCGGATTGG AGTCTGCAAC
TGGAGCGCTC TCGTTCGCCT GGAGTATTTC ACGCAGCATC AGGCCTAACC TCAGACGTTG

      1330       1340       1350       1360       1370       1380
TCGACTCCAT GAAGTCGGAA TCGCTAGTAA TCGTGGATCA GAATGCCACG GTGAATACGT
AGCTGAGGTA CTTCAGCCTT AGCGATCATT AGCACCTAGT CTTACGGTGC CACTTATGCA
                                                  GC CACTTATGCA       1743

      1390       1400       1410       1420       1430       1440
TCCCGGGCCT TGTACACACC GCCCGTCACA CCATGGGAGT GGGTTGCAAA AGAAGTAGGT
AGGGCCCGGA ACATGTGTGG CGGGCAGTGT GGTACCCTCA CCCAACGTTT TCTTCATCCA   1743
AGGGCCCGGA ACATG

      1450       1460       1470       1480       1490       1500
AGCTTAACCT TCGGGAGGGC GCTTACCACT TTGTGATTCA TGACTGGGGT GAAGTCGTAA
TCGAATTGGA AGCCCTCCCG CGAATGGTGA AACACTAAGT ACTGACCCCA CTTCAGCATT

      1510       1520       1530       1540       1550
CAAGGTAACC GTAGGGGAAC CTGCGGTTGG ATCACCTCCT TA.......
GTTCCATTGG CATCCCCTTG GACGCCAACC TAGTGGAGGA AT.......
```

FIGURE 89



```
E.colirrsE   530 GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTT
Cam.jejun5   506 GTAATACGGAGGGTGCAAGCGTTACTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGATT
Stp.aureus   538 GTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTT

E.colirrsE   592 GTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTT
Cam.jejun5   568 ATCAAGTCTCTTGTGAAATCTAATGGCTTAACCATTAAACTGCTTGGGAAACTGATAGTCTA
Stp.aureus   600 TTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTT

E.colirrsE   654 GAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA
Cam.jejun5   630 GAGTGAGGGAGAGGCAGATGGAATTGGTGGTGTAGGGGTAAAATCCGTAGATATCACCAAGA
Stp.aureus   662 GAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGA

E.colirrsE   716 ATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA
Cam.jejun5   692 ATACCCATTGCGAAGGCGATCTGCTGGAACTCAACTGACGCTAAGGCGCGAAAGCGTGGGGA
Stp.aureus   724 ACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGA

E.colirrsE   778 GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGC
Cam.jejun5   754 GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTACACTAGTTGTTGGGGT
Stp.aureus   786 TCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGG

E.colirrsE   840 C-CTTGA-GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGC
Cam.jejun5   816 G-CTAGT-CATCTCAGTAATGCAGCTAACGCATTAAGTGTACCGCCTGGGGAGTACGGTCGC
Stp.aureus   848 GT-TTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGC

E.colirrsE   900 AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Cam.jejun5   876 AAGATTAAAACTCAAAGGAATAGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Stp.aureus   909 AAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT

E.colirrsE   962 CGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAAT
Cam.jejun5   938 CGAAGATACGCGAAGAACCTTACCTGGGCTTGATATCCTAAGAACCTTTTAGAGATAAGAGG
Stp.aureus   971 CGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCC

E.colirrsE  1024 GTG--CCTTCGGG--AA-CCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA
Cam.jejun5  1000 GTGCTAGCTTGCTAGAA-CTTAGAGACAGGTGCTGCACGGCTGTCGTCAGCTCGTGTCGTGA
Stp.aureus  1033 TTCC-CCTTCGGG--GGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA

SB-1                                  GCAACGAGCGCAACCC
E.colirrsE  1081 AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGG-CC
Cam.jejun5  1061 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGTATTTAGTTGCTAACGGTTCGG-CC
Stp.aureus  1092 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCA-TTAAGT-T
```

FIGURE 89


```
                                                                    ATGACGTCAAGTCATC
SB-3  (SEQ ID NO:157)                                               ATGACGTCAAGTCATC
SB-4  (SEQ ID NO:154)
E.colirrsE 1142 GGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATC
Cam.jejun5 1122 GAGCACTCTAAATAGACTGCCTTCG-TAAGGAGGAGGAAGGTGTGGACGACGTCAAGTCATC
Stp.aureus 1152 GGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC


SB-3               ATGGCCCTTA
SB-4               ATGGCCCTTACGA
E.colirrsE 1204 ATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTC
Cam.jejun5 1183 ATGGCCCTTATGCCCAGGGCGACACACGTGCTACAATGGCATATAGAATGAGACGCAATACC
Stp.aureus 1214 ATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACC


E.colirrsE 1266 GCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTC
Cam.jejun5 1245 GCGAGGTGGAG-CAAATCTATAAAATATGTCCCAGTTCGGATTGTTCTCTGCAACTCGAGAG
Stp.aureus 1276 GCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTA


E.colirrsE 1328 CATGAAGTCGGAATCGCTAGTAATCGTGGATCAGA-ATGCCACGGTGAATACGTTCCCGGGC
Cam.jejun5 1306 CATGAAGCCGGAATCGCTAGTAATCGTAGATCAGCCATGCTACGGTGAATACGTTCCCGGGT
Stp.aureus 1338 CATGAAGCTGGAATCGCTAGTAATCGTAGATCAGC-ATGCTACGGTGAATACGTTCCCGGGT
                                                                    CGGTGAATACGTTCCCGGGC
1743(compl)


E.colirrsE 1389 CTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCT
Cam.jejun5 1368 CTTGTACTCACCGCCCGTCACACCATGGGAGTTGATTTCACTCGAAGCCGGAATACT--A-A
Stp.aureus 1399 ATTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCT
1743(compl)       CTTGTAC

E.colirrsE 1451 TCG-GGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCG
Cam.jejun5 1427 AC---T-AGTTACCGTCCACAGTGGAATCAGCGACTGGGGTGAAGTCGTAACAAGGTAACCG
Stp.aureus 1461 TTTAGGAGCTAGCCGTCGAAGGTGGGACAAATGATTGGGGTGAAGTCGTAACAAGGTAGCCG


E.colirrsE 1512 TAGGGGAACCTGCGGTTGGATCACCTCCTTA~~~
Cam.jejun5 1485 TAGGAGAACCTGCGGTTGGATCACCTCCT~~~~~
Stp.aureus 1523 TATCGGAAGGTGCGGCTGGATCACCTCCTTTCT~
```

FIGURE 90

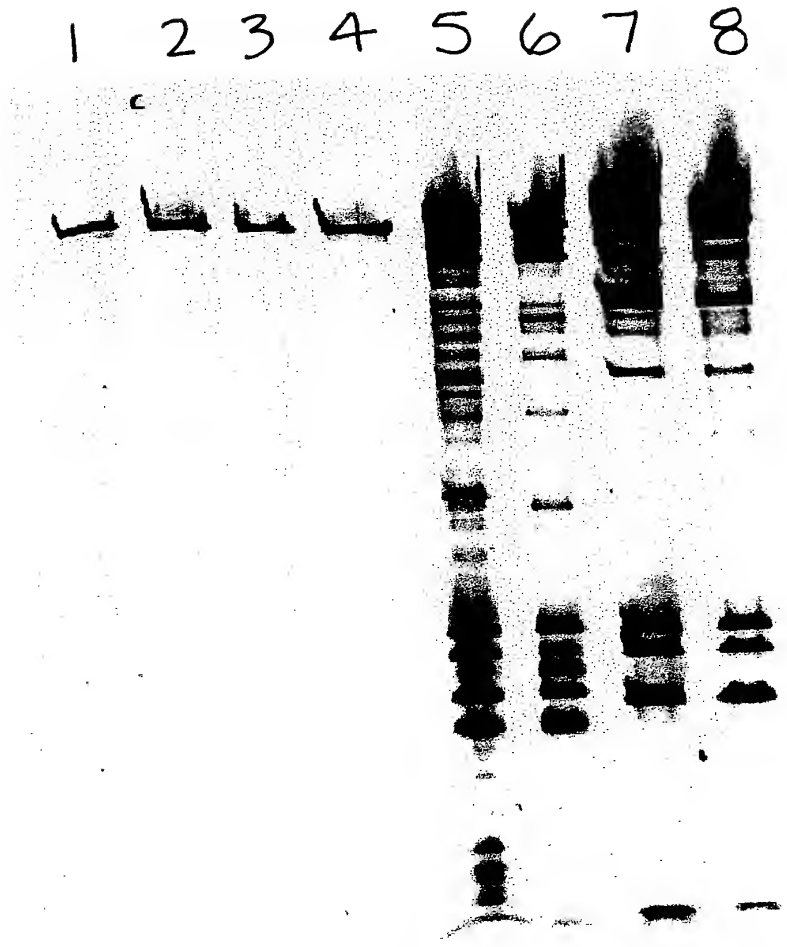


FIGURE 91

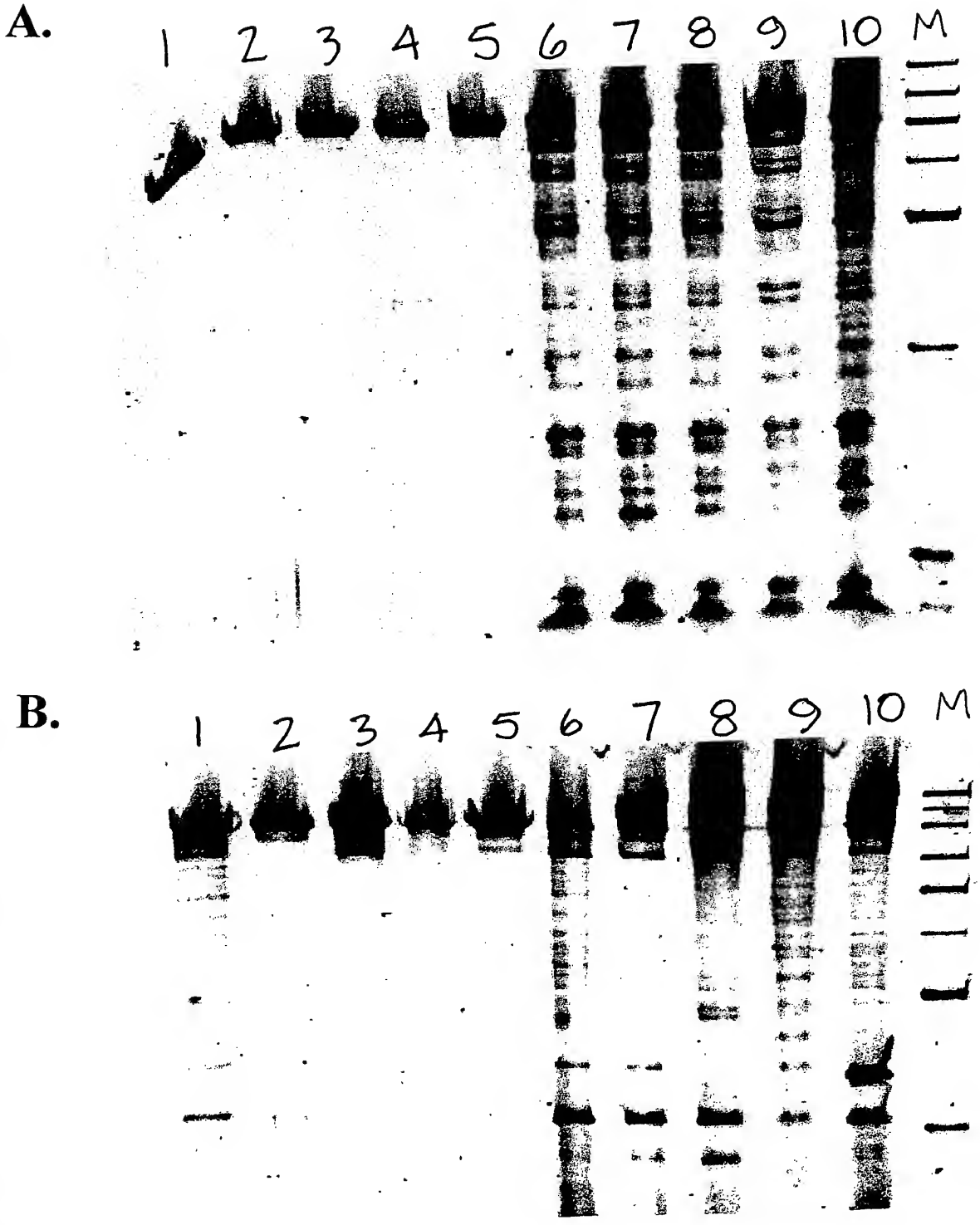


FIGURE 92

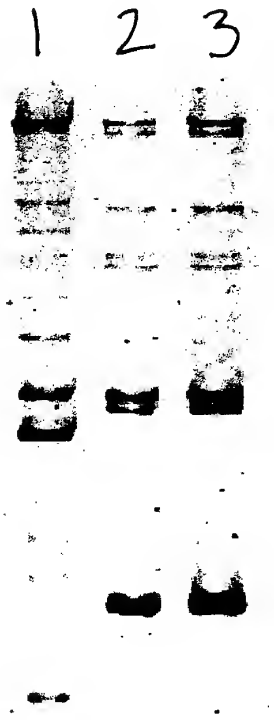


FIGURE 93

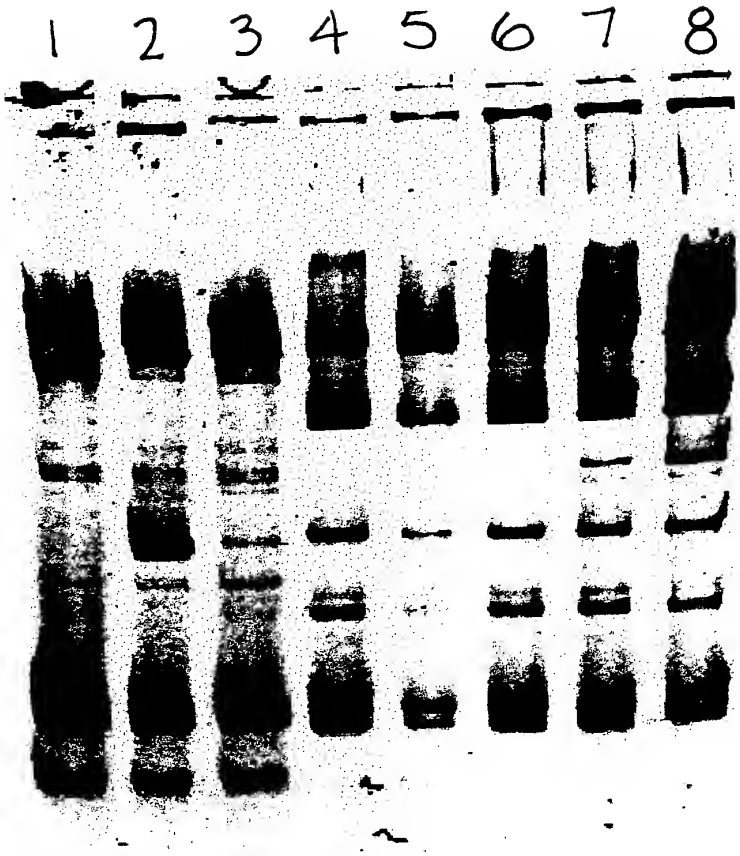
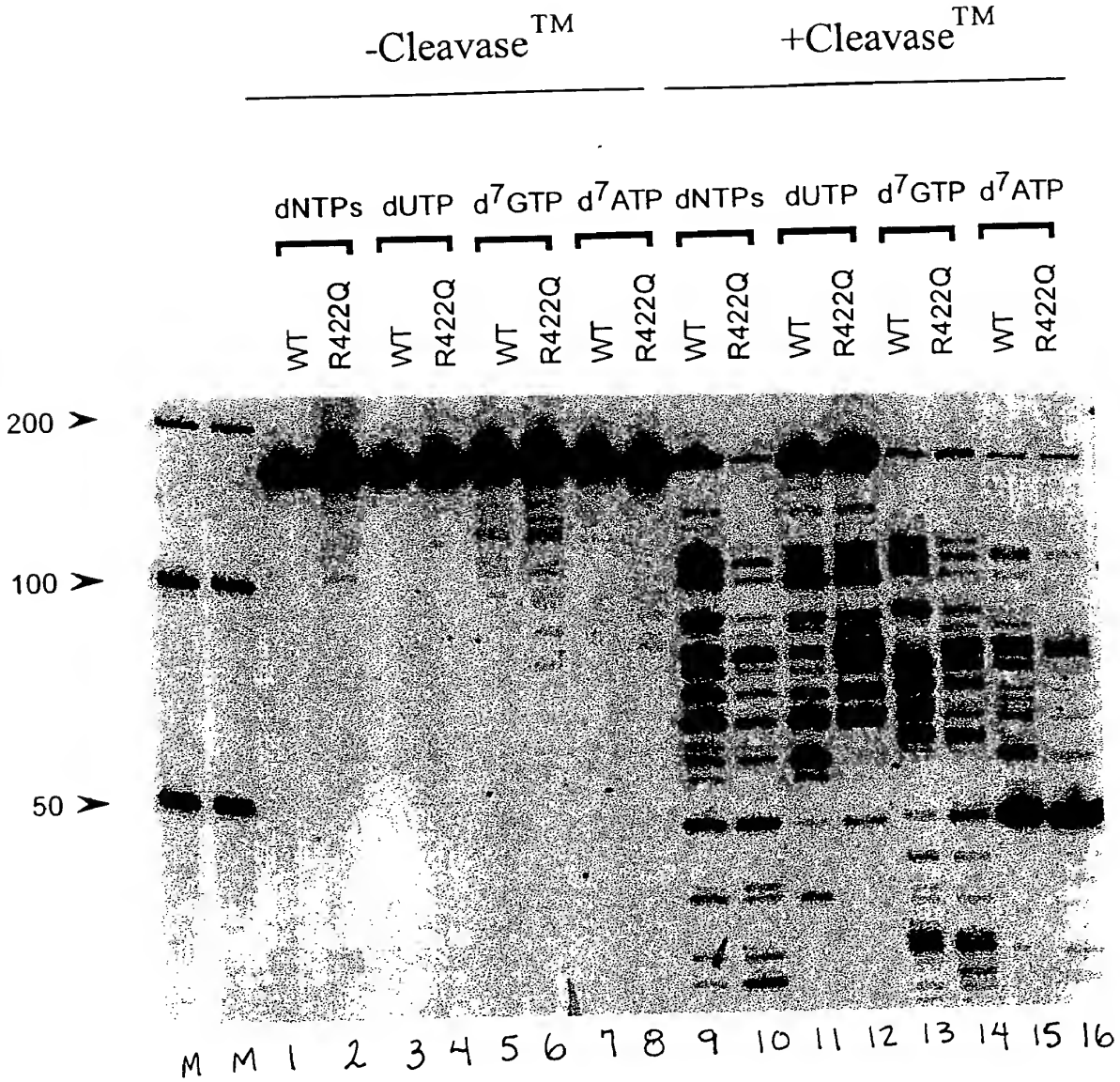


FIGURE 94


-Cleavase™
+Cleavase™
dNTPs
dUTP
d7GTP
d7ATP
dNTPs
dUTP
d7GTP
d7ATP
WT
R422Q
200
100
50
M M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16